(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 May 2002 (10.05.2002)

PCT

(10) International Publication Number
WO 02/36824 A1

(51) International Patent Classification[7]: C12Q 1/68, C12N 15/12, A01K 67/027, C07K 16/40

(21) International Application Number: PCT/NZ01/00245

(22) International Filing Date: 31 October 2001 (31.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

507888	31 October 2000 (31.10.2000)	NZ
508662	6 December 2000 (06.12.2000)	NZ

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/36824 A1

(54) Title: MARKER ASSISTED SELECTION OF BOVINE FOR IMPROVED MILK PRODUCTION USING DIACYLGLYCEROL ACYLTRANSFERASE GENE DGAT1

(57) Abstract: The present invention provides a method of genotyping bovine for improved milk production traits by determining the DGAT1 genotypic state of said bovine, wherein the DGAT1 gene and polymorphisms within said gene have been found to be associated with such improved milk production traits.

Marker Assisted Selection of Bovine for Improved Milk Production Using Diacylglycerol Acyltransferase gene DGAT1

FIELD OF THE INVENTION

This invention relates to an application of marker assisted selection of bovine for a quantitative trait loci (QTL) associated with milk production, particularly although by no means exclusively, by assaying for the presence of at least one allele which is associated with increased milk volume as well as improved milk composition. The present invention also relates to the gene associated with the QTL, various polymorphisms within the gene sequence, proteins encoded by these sequences as well as to the application of all of these in the farming industry.

BACKGROUND

The genetic basis of bovine milk production is of immense significance to the dairy industry. An ability to modulate milk volumes and content has the potential to alter farming practices and to produce products which are tailored to meet a range of requirements. In particular, a method of genetically evaluating bovine to select those which express desirable traits, such as increased milk production and improved milk composition, would be desirable.

To date, bovine genomics are poorly understood and little is known regarding the genes which are critical to milk production. While there have been reports of quantitative trait loci (QTLs) on bovine chromosome 14 postulated to be associated with milk production (Coppieters *et al* (1998)), the specific genes involved have not to date been identified.

Marker assisted selection, which provides the ability to follow a specific favourable genetic allele, involves the identification of a DNA molecular marker or markers that segregate with a gene or group of genes associated with a QTL. DNA markers have several advantages. They are relatively easy to measure and are unambiguous, and as DNA markers are co-dominant, heterozygous and homozygous animals can be distinctively identified. Once a marker system is established, selection decisions are able to be made very easily as DNA markers can be assayed at any time after a DNA containing sample has been collected from an individual infant or adult animal, or even earlier as it is possible to test embryos in vitro if such embryos are collected.

The applicants have now identified a gene responsible for the QTL effect on bovine chromosome 14 as well as a number of polymorphisms which are associated with distinct genetic merits of animals for milk composition and volume.

It is an object of the present invention to provide an application method for marker assisted selection of this bovine gene, and in particular, of the polymorphisms in the bovine gene which are associated with increased milk volume and altered milk composition; and/or to provide genetic markers for use in such a method; and/or to provide the nucleic acid and amino acid sequences of this gene and encoded polypeptide; and/or to provide animals selected using the method of the invention as well as milk produced by the selected animals; and/or to provide the public with a useful choice.

SUMMARY OF THE INVENTION

This invention relates to the discovery of the bovine Diacylglycerol-o-acyltransferase (DGAT1) gene and polymorphisms within the bovine DGAT1 gene which are associated with increased milk yield and altered milk composition.

More specifically, several polymorphisms in the bovine DGAT1 gene have been identified distinguishing multiple DGAT1 alleles in different cattle breeds. These polymorphisms include: K232A (Bases 6829/30 AA-CG nucleic acid change and K-A amino acid change); Nt984+8(Base 7438 A-G nucleic acid change); Nt984+26(Base 7456 C-T nucleic acid change); Nt1470+85(Base 8402 C-T nucleic acid change); Nt191+435 (Base 626 T-G nucleic acid change); Nt191-3321 (Base 3512 T-G nucleic acid change); Nt279+144 (Base 4040 T-C nucleic acid change); Nt279+1067 (Base 4963 A-G nucleic acid change); Nt279+1107 (Base 5003 G-A nucleic acid change); Nt358 (Base 5997 C-T nucleic acid change); Nt754+3 (Base 6892 G-A nucleic acid change); Nt897+32 (Base 7224/5 GG-AC nucleic acid change); Nt1251+42 (Base 7987 G-A nucleic acid change) as summarised in Table 1. In particular, DGAT1 alleles characterized by the K232A mutation have been identified as being associated with an increased milk volume and altered milk composition in animals dependent upon whether they are homozygous with or without the mutation or heterozygous carrying one mutated allele. More specifically, the presence of the K232A mutation results in a decrease in milkfat percentage, milkfat yield, solid fat content and milk protein percentage, while increasing milk volume and milk protein yield.

The present invention thus relates to the use of the polymorphisms in a method of identification and selection of a bovine having at least one of said polymorphisms as well as to providing markers specific for such identification. Kits comprising said markers for use in marker selection also form part of the present invention as do animals so selected, the milk produced by such selected animals and products produced from such milk, particularly as such milk and milk products affect processing and/or health characteristics for consumers.

In particular, the present invention is directed to a method of genotyping cows or bulls for one or more of the polymorphisms disclosed herein, selected cows or bulls so genotyped and milk and semen from said selected cows and bulls respectively.

According to a further aspect the present invention is directed to the isolated DGAT1 nucleic acid and allelic nucleic acid molecules comprising polymorphisms as well as to the proteins encoded thereby and their polypeptide sequences. Antibodies raised against said proteins are also contemplated, as are vectors comprising the nucleic acid molecules, host cells comprising the vectors; and protein molecules expressed in said host cells; and the application of all of them in the farming industry.

In particular, such applications include methods for modulating milk production and/or composition in a lactating bovine by affecting DGAT1 activity, by reducing the activity of DGAT1 (e.g. by use of specific ribozymes, antisense sequences and/or antibodies, or by transgenic technology to produce a "knock out" bovine and/or bovine with introduced transgenes containing the DGAT1 gene and/or variations of this gene driven by various promoters).

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described with reference to the Figures of the accompanying drawings in which:

Figure1: Shows a BAC contig spanning the *BULGE13-BULGE09* interval relative to a schematic diagram of bovine chromosome 14 and a schematic diagram showing the location of the genetic markers. The most likely position of the QTL is shown as a bar on the FISH-anchored linkage map proximal to BTA14q. The BACs composing the contigs spanning the *BULGE13-BULGE09* interval are shown as a series of horizontal lines. The symbols on each BAC indicate their individual STS content: solid circles

correspond to STS derived from BAC ends, open boxes to microsatellite markers, and solid triangles to gene-specific Comparative Anchored Tagged Sequences. The arrow heads mark the BACs from which the respective BAC end STS were derived. The length of the lines do not reflect the actual insert size of the corresponding BACs. The BAC contig was aligned with the orthologous human HSA8q24.3 genomic "golden path" sequence represented according to the Ensembl Human Genome Server (http://www.ensembl.org/): individual sequence contigs are shown in alternating light and dark; a horizontal line indicates a gap in the sequence assembly; genetic markers are indicated under the contig map; the lines and boxes above the contig map represent "curated", "predicted known" or "predicted novel" genes.

Figures 2a and 2b: Show the genomic sequence of the bovine DGAT1 gene. Figure 2a is the 31 base pair sequence upstream but adjacent to the ATG or translation start site and is 5'UTR. Figure 2b is the genomic sequence in the bovine DGAT1 gene from the ATG translation start site (base 1) through to genomic sequence flanking the gene at the 3' end. The significant features including intron/exon boundaries, polymorphic sites, polyadenylation signal, and alternate splicing site and some of the primer sequences used in the assays described herein, are indicated;

Figure 3: Shows the genomic organization, four polymorphisms and haplotypes found in the bovine *DGAT1* gene. Leader and trailer sequences are shown in light grey, coding sequences in dark grey and intronic sequences as a hollow line. The positions of four of the identified polymorphisms are marked as shown on the gene, and detailed in the underlying boxes including the corresponding sequence traces All the sequence variations are summarised in Table 1. The four *DGAT1* haplotypes which were found in the Dutch and New-Zealand Holstein-Friesian population as defined by these polymorphisms are shown and referred to as "sHQ^{-D}", "sHQ^{-NZ}" , "sHQ^{-III}" for the fat increasing haplotypes and "shq" for the fat decreasing haplotype;

Figure 4a: Shows the corresponding full length amino acid sequence for DGAT1 sequence of Figure 2b including annotation of the amino acid substitution;

Figure 4b: Shows the amino acid sequence predicted as a result of alternate splicing with exon VIII;

Figure 5: Shows the multiple peptide alignment of a portion of the *DGAT1* protein flanking the K232A substitution from *Bos taurus*, *Bison bison*, *Ovis aries*, *Sus scrofa*,

Homo sapiens, *Cercopithecus aethiops*, *Mus musculus domesticus* and *Rattus norvegicus* showing the evolutionary conservation of the lysine mutated in the bovine *K232A* polymorphism;

Figure 6: *A.* Shows the frequency distribution of observed *DGAT1* *SNP* haplotypes in the Dutch and New Zealand Holstein-Friesian dairy cattle populations. *B-D.* Shows the frequency distribution of the combined *microsatellite* (*BULGE09-BULGE11*) and *SNP* *DGAT1* haplotypes. The $H^{Q\text{-}D}$ and $H^{Q\text{-}NZ}$ haplotypes are shown; and

Figure 7: Shows the lod score due to LD when including (+) or excluding (-) the four DGAT1 polymorphisms shown in Figure 3 in a combined linkage and LD multipoint maximum likelihood mapping method. The lod score corresponds to the $\log_{10}$ of the ratio between the likelihood of the data assuming LD and linkage between the markers and the likelihood of the data assuming linkage in the absence of LD. The positions of the microsatellites and SNP markers utilized in the analysis are shown on the X-axis, while the position of the *DGAT1* SNPs is marked by a red arrow at the top of the figure.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered for the first time that the DGAT1 gene in bovine is associated with the QTL on chromosome 14 which is linked with improved milk production traits. More particularly, a number of novel polymorphisms on the DGAT1 gene have been discovered. It is thought that one or more of these polymorphisms is responsible for these traits.

The method used for isolating genes which cause specific phenotypes is known as positional candidate cloning. It involves: (i) the chromosomal localisation of the gene which causes the specific phenotype using genetic markers in a linkage analysis; and (ii) the identification of the gene which causes the specific phenotype amongst the "candidate" genes known to be located in the corresponding region. Most of the time these candidate genes are selected from available mapping information in humans and mice.

The tools required to perform the initial localisation (step (i) above) are microsatellite marker maps, which are available for livestock species and are found in the public domain (Bishop *et al.*, 1994; Barendse *et al.*, 1994; Georges *et al.*, 1995; and Kappes, 1997). The tools required for the positional candidate cloning, particularly the BAC libraries, (step (ii) above) are partially available from the public domain. Genomic

libraries with large inserts constructed with Bacterial Artificial Chromosomes (BAC) are available in the public domain for most livestock species including cattle. For general principles of positional candidate cloning, see Collins, 1995 and Georges and Anderson, 1996.

Recently, a quantitative trait locus (QTL) with major effect on milk solids composition, located at the centromeric end of bovine chromosome 14, has been reported (Coppieters *et al.*, (1998)). This QTL was shown to effect milk fat content and in particular to significantly affect protein %, volume, protein yield and fat yield of milk. The linkage study as well as subsequent marker assisted segregation analyses allowed for the identification of thirteen Holstein-Friesian sires predicted to be heterozygous "*Qq*" for the corresponding QTL (Coppieters *et al.*, (1998); Riquet *et al.*, (1999)).

Linkage disequilibrium methods were applied to refine the map position of the QTL to a ≈ 5 cM interval bounded by microsatellite markers BULGE09 and BULGE30.

A bovine DGAT1 nucleotide sequence was determined by the applicants and is shown in Figures 2a and 2b with the corresponding amino acid sequences (long and short forms) being shown in Figures 4a and 4b respectively. Table 1 sets out all the polymorphisms located to date with reference to the sequence in Figure 2b. Some of the genetic polymorphisms identified in the bovine DGAT1 gene are reported in Figure 3. The nucleic acid and protein sequences of the DGAT1 alleles including the K232A mutation are shown in Figures 2a and 2b (SEQ ID NOs: 3 and 1), annotated to show the alternatively spliced forms. The cDNA sequence is also set out in SEQ ID NO: 4.

The sequence information in the Figures gives rise to numerous, and separate, aspects of the invention.

In one aspect, the invention provides a method of determining genetic merit of a bovine with respect to milk composition and volume which comprises the step of determining the bovine DGAT1 genotypic state of said bovine. In particular, this method is useful for genotyping and selecting cows and bulls having the desired genotypic state so that milk and semen may be collected from said cows and bulls respectively. Such semen would be useful for breeding purposes to produce bovine having the desired genotypic and, as a result, phenotypic state. In addition, cows genotyped by the methods of the present invention are also useful for breeding purposes, particularly for breeding with the selected bulls and/or to be artificially inseminated with the semen from selected

bulls. The embryos and offspring produced by such cows also form part of the present invention.

In one embodiment, the genotypic state is determined with respect to DNA obtained from said bovine.

Alternatively, said genotypic state is determined with reference to mRNA obtained from said bovine.

In yet a further embodiment, the genotypic state is determined with reference to the amino acid sequence of expressed bovine DGAT1 protein obtained from said bovine.

Conveniently, in said method, the genotypic state of DNA encoding bovine DGAT1 is determined, directly or indirectly.

Alternatively, in said method the genotypic state of at least one nucleotide difference from the nucleotide sequence encoding bovine DGAT1 is determined, directly or indirectly.

More specifically, in said method the genotypic state of bovine DGAT1 allele(s) characterised by one or more of the polymorphisms shown in Table 1 below, is determined, directly or indirectly.

Table 1:
Table of polymorphisms in the bovine DGAT1 gene

Start codon (atg); the a residue is denoted as position 1

Base number relative to exonic sequence[1]	Nucleotide distance from start	substitution	Intron/exon #
Nt 191 + 435	626	T-G CAGTGCTAGGGG CAGTGCGAGGGG	Intron 1
Nt 191 + 3321	3512	T-G GCATTGCGCT GCATGGCGCT	Intron 1
Nt 279 + 144	4040	T-C TACCCTGGGAC TACCCCGGGAC	Intron 2
Nt 279 +1067	4963	A-G CTCTTAGCAGC CTCTTGGCAGC	Intron 2
Nt 279 +1107	5003	G-A ACAGGCAACT ACAGACAACT	Intron 2
Nt 358	5997	C-T TGTCTCTGTTC TGTCTTTGTTC	Exon IV
Nt 692	6829	AA-GC GGTAAGAAGGCCAA (Q) GGTAAGGCGGCCAA (q)	K232A Exon VIII *
Nt 754 +3	6892	G-A GCGGTGAGGAT GCGGTAAGGAT	Intron VIII
Nt 897 +32	7224	GG-AC GGGGGGGGGGGACTCT GGGGGACGGGGACTCT	Intron X
Nt 984 +8	7438	A-G GAGTGACCTGC GAGTGGCCTGC	Intron XII *
Nt 984 +26	7456	C-T GGACGCGTGGG GGACGTGTGGG	Intron XII *
Nt1251 +42	7987	G-A GGTGGGGGTGG GGTGGAGGTGG	Intron XV
Nt 1470 +85	8402	C-T CTGGGCGCAGC CTGGGTGCAGC	3' flanking region *

The numbers given are for the actual nucleotide or in the case of two nucleotide substitutions to the first nucleotide in the variation (counting 5' to 3')
*More detail of these polymorphisms is given in Figure 2b.
[1] e.g. Nt 191 represents nucletode number 191 from the start site of the coding sequence, + 435 represents number of nucleotides from and including base 192 in the genomic sequence (intron 1) to the polymorphic nucleotide
The polymorphic nucleotides are shaded

Preferably, the invention is directed to a method of determining the genotypic state of bovine DGAT1 allele(s) by determining the presence of the K232A polymorphism, either directly or indirectly.

There are numerous art standard methods known for determining whether a particular DNA sequence is present in a sample. An example is the Polymerase Chain Reaction

(PCR). A preferred aspect of the invention thus includes a step in which ascertaining whether a polymorphism(s) in the sequence of DGAT1 DNA is present, includes amplifying the DNA in the presence of primers based on the nucleotide sequence of the DGAT1 gene and flanking sequence, and/or in the presence of a primer containing at least a portion of a polymorphism as disclosed herein and which when present results in altered relative milk lipid and protein production, and milk volume.

A primer of the present invention, used in PCR for example, is a nucleic acid molecule sufficiently complementary to the sequence on which it is based and of sufficient length to selectively hybridise to the corresponding portion of a nucleic acid molecule intended to be amplified and to prime synthesis thereof under *in vitro* conditions commonly used in PCR. Likewise, a probe of the present invention, is a molecule, for example a nucleic acid molecule of sufficient length and sufficiently complementary to the nucleic acid molecule of interest, which selectively binds under high or low stringency conditions with the nucleic acid sequence of interest for detection thereof in the presence of nucleic acid molecules having differing sequences.

In another aspect, the invention provides a method for determining the genetic merit of bovine with respect to milk content and volume with reference to a sample of material containing mRNA obtained from the bovine. This method includes ascertaining whether a polymorphism(s) in the sequence of the mRNA encoding DGAT1 is present. The presence of such polymorphisms again indicates an association with altered relative milk lipid and protein production and milk volume.

Again, if an amplification method such as PCR is used in ascertaining whether a polymorphism(s) in the sequence of the mRNA encoding (DGAT1) is present, the method includes reverse transcribing the mRNA using a reverse transcriptase to generate a cDNA and then amplifying the cDNA in the presence of a pair of primers complementary to a nucleotide sequence encoding a protein having biological activity of wild type DGAT1.

In a further aspect, the invention includes the use of a probe in the methods of genotyping according to the invention wherein the probe is selected from any 5 or more contiguous nucleotides of the DGAT1 sequence as shown in Figure 2b, which is therefore sufficiently complementary with a nucleic acid sequence encoding such bovine DGAT1, or its complement, so as to bind thereto under stringent conditions. Diagnostic kits containing such a probe are also included. Such probes may be selected from

ForAA (FAM): CGTTGGCCTTCTTA or DgatADGC (VIC): TTGGCCGCCTTACC. (SEQ ID NOs: 20 and 21 respectively.)

The invention further includes isolated nucleic acid molecules encoding the DGAT1 variant proteins i.e. those proteins encoded by SEQ ID NOs: 1 and 4 (Figure 2b), with or without one or more polymorphisms of SEQ ID Nos: 7 to 19 (Table 1), or a fragment or variant thereof. Particularly, the invention includes an isolated nucleic acid molecule comprising a DNA molecule having in whole or in part the nucleotide sequence identified in Figure 2b or which varies from the sequence due to the degeneracy of the genetic code, or a nucleic acid strand capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

The invention includes isolated mRNA transcribed from DNA having a sequence which corresponds to a nucleic acid molecule of the invention.

The invention includes isolated DNA in a recombinant cloning vector and a prokaryotic or eukaryotic cell containing and expressing heterologous DNA of the invention.

The invention includes a transfected cell line which expresses a protein encoded by the nucleic acid molecules of the invention.

The invention also includes a primer composition useful for detection of the presence of DNA encoding DGAT1 and/or the presence of DNA encoding a variant protein. In one form, the composition can include a nucleic acid primer substantially complementary to a nucleic acid sequence encoding DGAT1. The nucleic acid sequence can in whole or in part be that identified in Figure 2b. Diagnostic kits including such a composition are also included.

The invention further provides a diagnostic kit useful in detecting DNA encoding a variant DGAT1 protein in bovine which includes first and second primers for amplifying the DNA, the primers being complementary to nucleotide sequences of the DNA upstream and downstream, respectively, of a polymorphism in the portion of the DNA encoding DGAT1 which results in altered relative milk lipid, solid fat content and protein production and milk volume, wherein at least one of the nucleotide sequences is selected to be from a non-coding region of the DGAT1 gene. The kit can also include a third primer complementary to a polymorphism, disclosed herein, located on the DGAT1 gene.

The invention includes a process for producing a protein of the invention, including preparing a DNA fragment including a nucleotide sequence which encodes the protein; incorporating the DNA fragment into an expression vector to obtain a recombinant DNA molecule which includes the DNA fragment and is capable of undergoing replication; transforming a host cell with the recombinant DNA molecule to produce a transformant which can express the protein; culturing the transformant to produce the protein; and recovering the protein from resulting cultured mixture.

Thus in a further aspect, the invention provides a purified protein having biological activity of DGAT1. The terms "isolated" and "purified" as used herein, each refer to a protein substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesised. In certain preferred embodiments, the protein having biological activity of DGAT1 comprises an amino acid sequence and variants shown in Figures 4a and 4b (SEQ ID NOs: 2, 5 and 6). Furthermore, proteins having biological activity of DGAT1 that are encoded by nucleic acids which hybridise under stringent conditions to a nucleic acid comprising a nucleotide sequence shown in Figure 2b (SEQ ID NOs: 1 and 4) are encompassed by the invention.

Proteins of the invention having DGAT1 activity can be obtained by expression of a nucleic acid coding sequence in a suitable host cell using techniques known in the art. Suitable host cells include prokaryotic or eukaryotic organisms or cell lines, for example, yeast, *E. coli*, insect cells and COS1 cells. The recombinant expression vectors of the invention can be used to express a protein having DGAT1 activity in a host cell in order to isolate the protein. The invention provides a method of preparing a purified protein of the invention comprising introducing into a host cell a recombinant nucleic acid encoding the protein, allowing the protein to be expressed in the host cell and isolating and purifying the protein. Preferably, the recombinant nucleic acid is a recombinant expression vector. Proteins can be isolated from a host cell expressing the protein and purified according to standard procedures of the art, including ammonium sulfate precipitation, column chromatography (eg. ion exchange, gel filtration, affinity chromatography, etc.) electrophoresis, and ultimately, crystallisation (see generally "Enzyme Purification and Related Techniques". *Methods in Enzymology*, 22, 233-577 (1971)).

Alternatively, the protein or parts thereof can be prepared by chemical synthesis using techniques well known in the chemistry or proteins such as solid phase synthesis (Merrifield, 1964), or synthesis in homogeneous solution (Houbenwcyl, 1987).

It will of course be understood that a variety of substitutions of amino acids is possible while preserving the structure responsible for activity of the DGAT1 proteins disclosed herein. Conservative substitutions are described in the patent literature, as for example, in United States Patent No 5,264,558 or 5,487,983. It is thus expected, for example, that interchange among non-polar aliphatic neutral amino acids, glycine, alanine, proline, valine and isoleucine, would be possible. Likewise, substitutions among the polar aliphatic neutral amino acids, serine, threonine, methionine, asparagine and glutamine could possibly be made. Substitutions among the charged acidic amino acids, aspartic acid and glutamic acid, could probably be made, as could substitutions among the charged basic amino acids, lysine and arginine. Substitutions among the aromatic amino acids, including phenylalanine, histidine, tryptophan and tyrosine would also likely be possible. These sorts of substitutions and interchanges are well known to those skilled in the art. Other substitutions might well be possible. Of course, it would also be expected that the greater percentage of homology ie. sequence similarity, of a variant protein with a naturally occurring protein, the greater the retention of activity.

A further advantage may be obtained through chimeric forms of the proteins, as known in the art. A DNA sequence encoding each entire protein, or a portion of the protein, could be linked, for example, with a sequence coding for the C-terminal portion of *E. coli* β-galactosidase to produce a fusion protein.

The proteins of the invention, or portions thereof, have numerous applications in turn. By way of example, each protein can be used to prepare antibodies which bind to a distinct epitope in an unconserved region of the protein. An unconserved region of the protein is one which does not have substantial sequence homology to other proteins.

Still further, the invention includes an antibody to a bovine DGAT1 variant protein encoded by a nucleotide sequence of the present invention as well as a diagnostic kit containing such an antibody.

Conventional methods can be used to prepare the antibodies. For example, by using a DGAT1 peptide, polyclonal antisera or monoclonal antibodies can be made using

standard methods. A mammal, (eg. a mouse, hamster, or rabbit) can be immunised with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunisation can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used to assess the levels of antibodies. Following immunisation, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunised animal and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalising these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (Kohler, 1975) as well as other techniques such as the human B-cell hybridoma technique (Kozbor, 1983) and screening of combinatorial antibody libraries (Huse, 1989). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide, and monoclonal antibodies isolated.

The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with the target protein. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Another method of generating specific antibodies, or antibody fragments, reactive against the target proteins is to screen expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria, with peptides produced from the nucleic acid molecules of the present invention. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries. See for example Ward *et al.*, Huse *et al.*, and McCafferty *et al.* (Ward, 1989); Huse 1989; McCafferty, 1990). Screening such libraries with, for example, a DGAT1 protein can identify immunoglobulin fragments reactive with that DGAT1. Alternatively, the SCID-hu mouse developed by Genpharm can be used to produce antibodies, or fragments thereof.

The polyclonal, monoclonal or chimeric monoclonal antibodies can be used to detect the proteins of the invention, portions thereof or closely related isoforms in various biological materials. For example, they can be used in an ELISA, radioimmunoassay or histochemical tests. Thus, the antibodies can be used to quantify the amount and location of a DGAT1 protein of the invention, portions thereof or closely related isoforms in a sample in order to determine the role of DGAT1 proteins. Using methods described hereinbefore, polyclonal, monoclonal antibodies, or chimeric monoclonal antibodies can be raised to non-conserved regions of DGAT1 and used to distinguish a particular DGAT1 from other proteins.

The polyclonal or monoclonal antibodies can be coupled to a detectable substance or reporter system. The term "coupled" is used to mean that the detectable substance is physically linked to the antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, and acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I; ^{131}I, ^{35}S and ^{3}H. In a preferred embodiment, the reporter system allows quantitation of the amount of protein (antigen) present.

Such an antibody-linked reported system could be used in a method for determining whether a fluid or tissue sample of a bovine contains a deficient amount or an excessive amount of the relevant DGAT1 protein. Given a normal threshold concentration of such a protein, test kits can be developed.

The availability of such antibodies gives rise to further applications. One is a diagnostic kit for identifying cells comprising an antibody (such as a monoclonal antibody) which binds to a protein comprising an amino acid sequence shown in Figure 4a and 4b; means for detecting the antibody when bound to the protein, unreacted protein or unbound antibody; means for determining the amount of protein in the sample; and means for comparing the amount of protein in the sample with a standard. In some embodiments of the invention, the detectability of the antibody which binds to a specific DGAT1 protein is activated by the binding (eg. change in fluorescence spectrum, loss of

radioisotopic label). The diagnostic kit can also contain an instruction manual for use of the kit.

Antibody-based diagnostics are of course not the only possibility. A further diagnostic kit comprises a nucleotide probe complementary to the sequence, or an oligonucleotide fragment thereof, shown in Figure 2a and 2b, for example, for hybridisation with mRNA from a sample of cells; means for detecting the nucleotide probe bound to mRNA in the sample with a standard. In a particular aspect, the kit of this aspect of the invention includes a probe having a nucleic acid molecule sufficiently complementary with a sequence identified in Figure 2a and 2b, or its complement, so as to bind thereto under stringent conditions. "Stringent hybridisation conditions" takes on its common meaning to a person skilled in the art. Appropriate stringency conditions which promote nucleic acid hybridisation, for example, 6x sodium chloride/sodium citrate (SSC) at about 45°C are known to those skilled in the art, including in Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989). Appropriate wash stringency depends on degree of homology and length of probe. If homology is 100%, a high temperature (65°C to 75°C) may be used. If homology is low, lower wash temperatures must be used. However, if the probe is very short (<100bp), lower temperatures must be used even with 100% homology. In general, one starts washing at low temperatures (37°C to 40°C), and raises the temperature by 3-5°C intervals until background is low enough not to be a major factor in autoradiography. The diagnostic kit can also contain an instruction manual for use of the kit.

One of the major applications of the present invention is in the marker assisted selection of bovines having a polymorphism in the DGAT1 gene and which are associated with improved milk production traits. The invention therefore provides a diagnostic kit which can be used to determine the DGAT1 genotype of bovine genetic material, for example. One kit includes a set of primers used for amplifying the genetic material. A kit can contain a primer including a nucleotide sequence for amplifying a region of the genetic material containing one of the polymorphisms described herein. Such a kit could also include a primer for amplifying the corresponding region of the normal DGAT1 gene, i.e. the sequence without polymorphisms. Usually, such a kit would also include another primer upstream or downstream of the region of interest complementary to a coding and/or non-coding portion of the gene. These primers are used to amplify the segment containing the mutation, i.e. polymorphism, of interest.

In particular, the invention is directed to the use of the polymorphisms in the DGAT1 gene in the genotyping of cows and bulls as well as to cows and bulls selected by such genotyping which have one or more of said polymorphisms in the DGAT1 gene. Such bulls so selected are of valuable breeding stock and the invention is also directed to the semen produced by such selected bulls for breeding purposes. Cows so selected are also useful as breeding stock as are their offspring. In addition, such cows may produce valuable dairy herds as the milk produced by such cows is produced in greater volumes than equivalent non-selected cows, and/or has an altered composition in that it comprises less milkfat and more milk protein. Such milk and products made therefrom also form part of the invention. It is also noted that the milk from these selected cows will be valuable as the fat content is not only decreased but is also characterised by being softer. Without being bound by theory, it is thought that this increased fat softness is due to the fatty acid composition being such that there is less saturated and more unsaturated fat in the milk of selected cows. Thus it is anticipated that products made from such milk will have processing advantages, such as in the production of more spreadable butter, as well as having a health benefit on consumers, as generally unsaturated fats are considered to be more "healthy" than saturated fats. The protein composition of milk produced by such selected cows is also altered. In particular, such milk comprises an altered protein yield compared to milk for non-selected cows and the casein:whey ratio is also altered which makes such milk valuable for cheese production.

Thus, the present invention involves genotyping bovine, both cows and bulls, for the DGAT1 polymorphisms disclosed herein, selected cows and bulls so genotyped, milk and semen produced by the selected cows and bulls so genotyped, offspring produced by the selected bovine, including embryos and cells (including cell lines) useful for cloning said selected bovine.

The actual genotyping is carried out using primers that target specific polymorphisms as described herein and that could function as allele-specific oligonucleotides in conventional hybridisation, Taqman assays, OLA assays, etc. Alternatively, primers can be designed to permit genotyping by microsequencing.

One kit of primers can include first, second and third primers, (a), (b) and (c), respectively. Primer (a) is based on a region containing a DGAT1 mutation such as described above. Primer (b) encodes a region upstream or downstream of the region to be amplified by primer (a) so that genetic material containing the mutation is amplified,

by PCR, for example, in the presence of the two primers. Primer (c) is based on the region corresponding to that on which primer (a) is based, but lacking the mutation. Thus, genetic material containing the non-mutated region will be amplified in the presence of primers (b) and (c). Genetic material homozygous for the DGAT1 gene will thus provide amplified products in the presence of primers (b) and (c). Genetic material homozygous for the mutated gene will thus provide amplified products in the presence of primers (a) and (b). Heterozygous genetic material will provide amplified products in both cases.

The present invention also contemplates the modulation of milk production and content in non-human animals by modulating the activity of the DGAT1 protein. In particular, this aspect of the invention includes a method of modulating milk production and/or milk content in a lactating bovine, the method comprising administering to the bovine an effective amount of a nucleic acid molecule substantially complementary to at least a portion of mRNA encoding the bovine DGAT1 variant proteins and being of sufficient length to sufficiently reduce expression of said DGAT1, i.e. by use of antisense nucleic acids.

Antisense nucleic acids or oligonucleotides (RNA or preferably DNA) can be used to inhibit DGAT1 production in a bovine if this is considered desirable e.g. in order to produce a bovine capable of improved milk production, i.e. increased milk volume and decreased milkfat content. Antisense oligonucleotides, typically 15 to 20 bases long, bind to the sense mRNA or pre mRNA region coding for the protein of interest, which can inhibit translation of the bound mRNA to protein. The cDNA sequence encoding DGAT1 can thus be used to design a series of oligonucleotides which together span a large portion, or even the entire cDNA sequence. These oligonucleotides can be tested to determine which provides the greatest inhibitory effect on the expression of the protein (Stewart 1996). The most suitable mRNA target sites include 5'- and 3'-untranslated regions as well as the initiation codon. Other regions might be found to be more or less effective.

Alternatively, an antisense nucleic acid or oligonucleotide may bind to DGAT1 coding sequences.

In yet another embodiment, the invention provides a method of modulating milk production and/or milk content in a lactating bovine, including administering to the bovine an effective amount of a nucleic acid molecule having ribozyme activity and a

nucleotide sequence substantially complementary to at least a portion of mRNA encoding a bovine DGAT1 and being of sufficient length to bind selectively thereto to sufficiently reduce expression of said DGAT1.

Rather than reducing DGAT1 activity in the bovine by inhibiting gene expression at the nucleic acid level, activity of the relevant DGAT1 protein may be directly inhibited by binding to an agent, such as, for example, a suitable small molecule or a monoclonal antibody.

Thus, the invention also includes a method of inhibiting the activity of bovine DGAT1 in a lactating bovine so as to modulate milk production and/or milk solids content, comprising administering an effective amount of an antibody to the relevant DGAT1.

The invention still further includes a method of modulating milk production and/or milk solids content by raising an autoantibody to a bovine DGAT1 in the bovine. Raising the autoantibody can include administering a protein having DGAT1 activity to the bovine.

In still a further embodiment, nucleic acids which encode DGAT1 proteins can be used to generate transgenic animals. A transgenic animal (eg. a mouse) is an animal having cells that contain a transgene, which transgene is introduced into the animal or an ancestor of the animal at a prenatal, eg. an embryonic stage. A transgene is DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, a bovine cDNA, comprising the nucleotide sequence shown in Figure 2b, or an appropriate variant or subsequence thereof, can be used to generate transgenic animals that contain cells which express the relevant DGAT1. Likewise, variants can be used to generate transgenic animals. "Knock out" animals can also be generated.

Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art are described, for example, in US Patent Nos. 4,736,866 and 4,870,009. In such methods, plasmids containing recombinant molecules are microinjected into mouse embryos. In particular, the plasmids can be microinjected into the male pronuclei of fertilised one-cell mouse eggs; the injected eggs transferred to pseudo-pregnant foster females; and the eggs in the foster females allowed to develop to term. (Hogan, 1986). Alternatively, an embryonal stem cell can be transfected with an expression vector comprising nucleic acid encoding a DGAT1 protein, and cells containing the nucleic acid can be used to form aggregation chimeras with embryos

from a suitable recipient mouse strain. The chimeric embryos can then be implanted into a suitable pseudopregnant female mouse of the appropriate strain and the embryo brought to term. Progeny harbouring the transfected DNA in their germ cells can be used to breed uniformly transgenic mice.

Such animals could be used to determine whether a sequence related to an intact DGAT1 gene retains biological activity of the encoded DGAT1. Thus, for example, mice in which the murine DGAT1 gene has been knocked out and containing the nucleic acid sequence identified in Figure 2b or fragment or variant thereof could be generated. The animals could be examined with reference to milk production and content.

The pattern and extent of expression of a recombinant molecule of the invention in a transgenic mouse is facilitated by fusing a reporter gene to the recombinant molecule such that both genes are co-transcribed to form a polycistronic mRNA. The reporter gene can be introduced into the recombinant molecule using conventional methods such as those described in Sambrook *et al.*, (Sambrook, 1989). Efficient expression of both cistrons of the polycistronic mRNA encoding the protein of the invention and the reporter protein can be achieved by inclusion of a known internal translational initiation sequence such as that present in poliovirus mRNA. The reported gene should be under the control of the regulatory sequence of the recombinant molecule of the invention and the pattern and extent of expression of the gene encoding a protein of the invention can accordingly be determined by assaying for the phenotype of the reporter gene. Preferably the reporter gene codes for a phenotype not displayed by the host cell and the phenotype can be assayed quantitatively. Examples of suitable reporter genes include lacZ (β-galactosidase), neo (neomycin phosphotransferase), CAT (chloramphenicol acetyltransferase) dhfr (dihydrofolate reductase), aphIV (hygromycin phosphotransferase), lux (luciferase), uidA (β-glucuronidase). Preferably, the reporter gene is lacZ which codes for β-galactosidase. β-galactosidase can be assayed using the lactose analogue X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) which is broken down by β-galactosidase to a product that is blue in colour.

Still further transgenic applications of the invention arise from knocking out the endogenous gene encoding DGAT1 in mammals and replacing this with a transgene, in order to obtain a desired effect. This is particularly true in cattle raised for milk production. For example, additional copies of the gene encoding DGAT1 can be inserted as a transgene, or the endogenous gene associated with a high level expression promoter in a transgene. It may also prove advantageous to substitute a defective gene

rather than delete the entire sequence of DNA encoding for a protein having DGAT1 activity. A method of producing a transgenic bovine or transgenic bovine embryo is described in United States Patent No. 5,633,076, issued 27 May 1997, for example.

These transgenic animals of the invention can again be used to investigate the molecular basis of DGAT1 action. For example, it is expected that mutants in which one or more of the conserved cysteine residues has been deleted would have diminished activity in relation to a DGAT1 protein in which all such residues are retained. Further, deletion of a proteolytic cleavage site would likely result in a mutant lacking biological activity of DGAT1.

Transgenic animals of the invention can also be used to test substances for the ability to prevent, slow or enhance DGAT1 activity. A transgenic animal can be treated with the substance in parallel with an untreated control transgenic animal. Substances which could be tested in this way include proteins extracted from foods ingested by the animal. For example, proteins extracted from pastoral grasses and other fodder can be tested to determine their effect on DGAT1 activity, including to determine whether breed-specific effects can be induced.

Thus, in further aspects, the invention provides transgenic non-human animals. These include by way of example only a transgenic bovine having a genome lacking a gene encoding a protein having biological activity of DGAT1 (or indeed any DGAT1 activity at all); a transgenic mouse having a genome containing a gene encoding a bovine protein having biological activity of any DGAT1; and a transgenic bovine having a gene encoding a bovine protein having biological activity of a bovine DGAT1 and heterologous nucleotide sequence antisense to the gene. The transgenic bovine can include a gene encoding a nucleic acid sequence having ribozyme activity and in transcriptional association with the nucleotide sequence antisense to the gene.

The invention further provides a transgenic bovine having a genome which includes additional copies of a gene encoding a protein having biological activity of DGAT1 or copies of a gene encoding a protein having biological activity of DGAT1 under control of a high expression promoter.

These are but a selection of the applications of this invention. Others will be apparent to those persons skilled in this art and are in no way excluded. To the contrary, the

invention extends to cover not only the specific teaching provided but also all variations and modifications which are within the skill and contemplation of the addressee.

The invention will now be defined by specific examples which are illustrative only and are not intended to limit the invention in any way.

EXPERIMENTAL

1. Location of the gene responsible for the observed QTL

Construction of a BAC contig spanning the BULGE9-BULGE30 interval.

In order to clone the gene(s) responsible for the observed QTL effect, a BAC contig spanning the corresponding marker interval was constructed. This was accomplished by screening a BAC library by filter hybridisation with the microsatellite markers available for proximal BTA14q, as well as with human cDNA clones mapping to the orthologous chromosome segment on the human RH transcript map: 8q23.3-ter (Riquet *et al.*, (1999)). The ends of the isolated BACs were sequenced, sequence tagged sites (STS) developed from the corresponding sequences, and mapped onto a bovine x hamster whole genome radiation hybrid panel. This STS content mapping approach lead to the construction of the BAC contig shown in Figure 1.

DGAT1 maps to the BULGE9-BULGE30 interval and is a strong positional candidate for the QTL.

A murine gene encoding a protein with Diacylglycerol-o-acyltransferase (DGAT1) activity was identified (Cases *et al.*, (1998)) and shown to completely inhibit lactation when knocked out in the mouse (Smith *et al.*, (2000)). This gene was reported in the human to map to HSA8qter (Cases *et al.*, (1998)), ie. in the region orthologous to that containing the bovine QTL. Screening the publicly available databases with the published murine and human DGAT1 cDNA sequences allowed identification of (i) a human BAC clone containing the human DGAT1 gene (AF205589), and (ii) three bovine Expressed Sequence Tags (AW446908; AW446985; AW652329) jointly covering approximately two thirds of the bovine gene. Aligning the human DGAT1 genomic sequences with the human and bovine cDNA sequences allowed the corresponding intron-exon boundaries to be identified. Primers were developed to PCR amplify a portion of the bovine DGAT1 gene. Screening the BACs composing the BULGE9-BULGE30 contig clearly indicated that the bovine DGAT1 gene was contained in a

subset of the BACs allowing us to accurately position the DGAT1 gene in the contig of Figure 1.

These results demonstrated that the map position of DGAT1 coincided with the most likely position of the chromosome 14 QTL as determined by linkage and linkage disequilibrium analyses. Knowing that the QTL primarily affects fat content, knowing the enzymatic activity of DGAT1 and the effect of a DGAT1 knock-out on lactation, this gene was considered to be a very strong positional candidate for the corresponding QTL.

Organisation of the bovine DGAT1 gene

The organisation of the bovine DGAT1 gene was determined by sequence analysis of one of the DGAT1 containing BACs. Primers were designed based on the available bovine, murine and human cDNA sequences which were either used for direct sequencing of the BAC clone or to generate PCR products corresponding to different parts of the bovine DGAT1 gene from this BAC which were then subjected to cycle-sequencing. All available sequences were then merged using the Phred / Phrap software (Ewing *et al.*, (1998); Ewing & Green, (1998); Gordon *et al.*, (1998)) to yield the consensus sequence shown in Figures 2a and b.

RT-PCR, 5' and 3' RACE experiments were performed on mRNA isolated from bovine mammary gland and the obtained PCR products subjected to cycle sequencing. Comparison of the genomic and cDNA sequences showed that the bovine *DGAT1* gene spans 8.6 Kb and comprises 17 exons measuring 121.8 bp on average (range: 42 – 436 bp) and allowed intron-exon boundaries to be identified (Figures 2a, 2b and 3). The cDNA sequence is also set out in SEQ ID NO: 4. While the first two introns are respectively 3.6 and 1.9 Kb long, the remaining 14 introns are only 92.4 bp long on average (range: 70 – 215 bp). All introns conform to the GT-AG rule and are strictly conserved between human and bovine. The bovine *DGAT1* gene is transcribed in a mRNA comprising >31 bp of 5' UTR sequence (Figure 2a), 1470 bp coding for a protein of 489 amino-acids, and 275 bp of 3' UTR sequence including a canonical AATAAA polyadenylation signal. The human and bovine *DGAT1* nucleotide (coding) and protein sequences are respectively 89.5% and 92.5% identical (Figures 2a, 2b, 4a and 4b). In addition, an alternative splicing variant is predicted in the bovine for exon VIII (Figure 2b). The corresponding bovine cDNAs are predicted to encode proteins comprising respectively 489 and 467 (alternative splicing variant) amino-acid residues (Figures 4a and 4b).

The predicted "Q" and "q" QTL alleles differ by a non conservative lysine to alanine amino-acid substitution in the DGAT1 gene.

Assuming that DGAT1 is indeed the QTL, it is predicted that the identified "*Q*" and "*q*" QTL alleles will correspond to functionally distinct DGAT1 alleles, ie. will differ at one or more mutations causing these alleles to be functionally different. To test this hypothesis, the structure of the DGAT1 gene in individuals predicted to be of different QTL genotypes: "*QQ*", "*Qq*" and "*qq*" was examined. More specifically, the DGAT1 gene from:

(i) two sires with "$H^{Q\text{-}D}/h^{q}$" genotype as well as two of their "$H^{Q\text{-}D}/H^{Q\text{-}D}$" offspring, two of their "h^{q}/h^{q}" offspring and one "$H^{Q\text{-}D}/h^{q}$" offspring, and

(ii) one "$H^{Q\text{-}NZ}/h^{q}$" sire with one of its "$H^{Q\text{-}NZ}/H^{Q\text{-}NZ}$" offspring

was analysed wherein $H^{Q\text{-}D}$ corresponds to the Dutch Q haplotype and $H^{Q\text{-}NZ}$ corresponds to the New Zealand Q haplotype, and primer pairs were designed that allowed for the amplification from genomic DNA of (i) the coding portion of exon I, (ii) exon II, and (iii) the chromosome regions spanning exons III to XVII. The corresponding PCR products from the selected individuals were cycle-sequenced and the resulting sequences examined with the *Polyphred* software.

Additional sequencing analysis, as described above, on DNA from a range of breeds revealed additional polymorphisms included in Table 1 (see Methods section for breeds). Four such polymorphisms were investigated further:

(i) K232A: a substitution of a *ApA* by a *GpC* dinucleotide in exon VIII (respectively positions 694 and 695 counting from the start codon in the cDNA). The substitution of these two adjacent nucleotides results in a non conservative lysine (hydrophylic basic amino acid) to alanine (hydrophobic amino acid) substitution in the DGAT1 protein. The *lysine* residue affected by this polymorphism is conserved in the human and murine DGAT1 sequences. Together with the resulting change in the electrical charge of the protein, this strongly suggests that this amino-acid substitution is likely to result in a functional difference between the two corresponding alleles and to be at least partly responsible for the observed QTL effect.

(ii) Nt984+8(Base 7438 A-G): A *A* to *G* substitution in intron 12, eight base pairs downstream of exon XII. Following standard nomenclature, this polymorphism will be

referred to as *Nt984+8(A-G)*. This polymorphism cannot be predicted as such to modify the functionality of the corresponding alleles although an effect on the splicing mechanism cannot be excluded given its proximity to the intron-exon boundary.

(iii) Nt984+26(Base 7456 C-T): A *C* to *T* substitution in intron 12, 26 base pairs downstream of exon XII. Following standard nomenclature, this polymorphism will be referred to as *Nt984+26(Base 7456 C-T)*. Again, this polymorphism cannot be predicted as such to modify the functionality of the corresponding alleles although an effect on the splicing mechanism cannot be excluded given its proximity to the intron-exon boundary.

(iv) Nt1470+85(Base 8402 C-T): A *C* to *T* substitution in the 3' UTR. Following standard nomenclature, this polymorphism will be referred to as *Nt1470+85(Base 8402 C-T)*. Again, this polymorphism cannot be predicted as such to modify the functionality of the corresponding alleles although an effect on polyadenylation or mRNA stability cannot be excluded.

Conclusion

These four polymorphisms were shown to assort into three distinct *SNP* haplotypes referred to as $sH^{Q\text{-}D}$, $sH^{Q\text{-}NZ}$ and sh^{q} because in the sequenced samples they coincided respectively with *microsatellite* haplotypes $\mu H^{Q\text{-}D}$, $\mu H^{Q\text{-}NZ}$ and μh^{q}. The base pair compositions of these three SNP haplotypes are shown in Figure 3.

Because the $sH^{Q\text{-}NZ}$ and sh^{q} marker haplotypes share the G residue at the *DGAT1 Nt984+8(Base 7438 A-G)* site, the causality of this polymorphism in the determinism of the QTL could be excluded. For the three remaining polymorphic sites, however, the *DGAT1* haplotypes associated with marker haplotypes $sH^{Q\text{-}D}$ and $sH^{Q\text{-}NZ}$ proved identical to each other while different from the sh^{q} *DGAT1* haplotype. Either of these three polymorphisms could therefore be responsible for the observed QTL effect. The *Nt984+26(Base 7456 C-T)* and *Nt1470+85(Base 8402 C-T)* polymorphisms are *a priori* more likely to be neutral with respect to *DGAT1* activity because of their respective location in an intron and the 3' UTR and likewise the other non coding or neutral polymorphism shown in Table 1. A direct effect of the *K232A* mutation on *DGAT1* activity, however, is very plausible. Indeed, the corresponding lysine residue is conserved amongst all examined mammals (i.e. human, mouse, rat, pig, sheep, bison) demonstrating its functional importance (Figure 5). The evolutionary conservation of

this lysine residue also demonstrates that the *K* residue characterizing the *sHQ-D* and *sHQ-NZ* marker haplotypes is more than likely the ancestral state and that it is the *A* residue characterizing the *shq* haplotypes that corresponds to a more recently evolved state.

2. Genotype Testing and Analysis I

This summarises the genotype testing and subsequent analysis of Holstein-Friesian animals sourced from New Zealand and Holland which were tested for the presence of the K232A polymorphism. Reference to allele "Q" corresponds to the K residue and allele "q" to the A residue (as shown in Figure 3 and Table 1).

An oligonucleotide ligation assay (OLA) was developed as described in the method section below that allows for efficient genotyping of the four *DGAT1* polymorphisms simultaneously. This OLA-test was used to genotype a previously described (Farnir et. al., 2000) "grand-daughter design" (i.e. series of paternal half-brother pedigrees) comprising 1,818 Dutch Holstein-Friesian sires as well as a "daughter design" (i.e. series of paternal half-sister pedigrees) comprising 529 New Zealand Holstein-Friesian cows selected according to phenotype as described below. The marker linkage phase for each individual was determined as described below.

Fig. 6 summarizes the frequency distribution of *DGAT1* haplotypes encountered in the Dutch and New Zealand populations respectively. Four distinct *SNP* haplotypes were identified. Three of these correspond to the *sHQ-D*, *sHQ-NZ* and *shq* that were previously identified by sequencing, and jointly account for 99% and 98% of the chromosomes in the Dutch and New-Zealand populations respectively. A fourth minor haplotype was found accounting for the remaining 1% and 2% of the chromosomes. As this haplotype codes for a *K* residue at position 232 it was assumed to correspond to a fat increasing "*Q*" allele and was therefore referred to as *sHQ-III* (Fig. 3). The observation that the *K* residue is found on three distinct *DGAT1* haplotypes while the *A* residue is found on a unique *DGAT1* haplotype is in agreement with *K* being the more ancient state.

The *sHQ-D* and *sHQ-NZ* SNP haplotypes (coding for a *K* residue at position 232) appear to be in strong linkage disequilibrium (LD) with the flanking microsatellite markers *BULGE09* and *BULGE11*, as they are in essence associated with unique microsatellite haplotypes corresponding respectively to the previously defined *μHQ-D* and *μHQ-NZ* haplotypes (Fig. 6C&D). In sharp contrast, the *shq* haplotype (coding for an *A* residue at

position 232) is nearly evenly distributed across more than ten distinct microsatellite haplotypes (Fig. 6B).

These observations are in excellent agreement with the results of the combined linkage and LD analysis (Fernier et. al., 2000). These studies indeed predicted (i) that in the Dutch population the vast majority (estimates ranging from 81% to 92%) of "*Q*" allele (= *K*) would reside on the μHQ^{-D} microsatellite haplotype, (ii) that in the New Zealand population a large fraction (estimates ranging from 36% to 51%) of "*Q*" alleles would reside on haplotype μHQ^{-NZ} (we now see that the remainder correspond mainly to the μHQ^{-D} microsatellite haplotype) and (iii) that in both populations the "*q*" alleles (= *A*) would correspond to multiple marker haplotypes, corresponding to hq.

Figure 7 illustrates the gain in LD signal that could be obtained in the Dutch Holstein-Friesian grand-daughter design when adding the *DGAT1* polymorphisms to the previously available markers for proximal BTA14q and performing a joint linkage and LD multipoint analysis (Fernier et. al., 2000) using the sires "daughter yield deviations" (DYD (Van Raden and Wiggans, 1991) corresponding to half breeding values) for milk fat percentage as phenotype. It can be seen that the lod score attributable to LD essentially doubles (from 3.7 to 7.8), and maximizes exactly at the position of the *DGAT1* gene. This result strongly supports the causal involvement of the *DGAT1* gene in the QTL effect. The corresponding ML estimates of the "*Q*" to "*q*" allele substitution effect ($\alpha/2$) (as defined in Falconer and Mackay, 1996), residual standard deviation (σ), population frequency of the "Q" allele (f_Q), number of generations to coalescence (g) and heterogeneity parameter (ρ) were respectively 0.11% ($\alpha/2$), 0.06% (σ), 0.20 (f_Q), 5 (g) and 0.84 (ρ).

Using the same Dutch Holstein-Friesian population, the additive effect of the *DGAT1* *K232A* polymorphism on milk yield and composition was examined. The sons DYDs for milk yield (kgs), protein yield (kgs), fat yield (kgs), protein percentage and fat percentage, were analysed using a mixed model including (i) a regression on the number of *K* alleles in the genotype (0, 1 or 2), and (ii) a random polygenic component estimated using an individual animal model and accounting for all known pedigree relationships. Table 2 below, reports the obtained results. It can be seen that the *K232A* mutation has an extremely significant effect on the five analysed dairy traits. The proportion of the trait variance explained by this polymorphism in this population ranges from 8% (protein yield) to 51% (fat percentage), corresponding to between 10% (protein yield) and 64% (fat percentage) of the genetic variance (= QTL + polygenic).

Note that the proportion of the variance explained by the full model ($1-r^2_{error}$) is of the order of 70% for the yield traits and 80% for the percentage traits, which is in agreement with the known reliabilities of the corresponding DYDs (Van Raden and Wiggans, 1991). An interesting feature of this QTL effect is that the "*q*" to "*Q*" substitution increases fat yield, while decreasing milk and protein yield, despite the strong overall positive correlation characterizing the three yield traits.

Table 2:

Effect of the *DGAT1 K232A* mutation on sire's daughter yield deviations (DYDs) for milk yield and composition.

Trait	α/2	r^2_{QTL}	p-value $_{QTL}$	$r^2_{polygenic}$	r^2_{error}
Milk yield (Kgs)	-158 Kgs	0.18	5.00E-35	0.49	0.32
Fat yield (Kgs)	5.23 Kgs	0.15	1.57E-29	0.55	0.30
Protein yield (Kgs)	-2.82 Kgs	0.08	1.70E-15	0.65	0.26
Fat %	0.17 %	0.51	4.33E-122	0.29	0.19
Protein %	0.04 %	0.14	5.05E-28	0.66	0.20

(i) α/2: QTL allele substitution effect on DYD (half breeding value), corresponding in the mixed model to the regression coefficient on the number of *K* alleles in the *DGAT1 K232A* genotype, and to α/2, where α is defined according to ref. Falconer and Mackay, 1996. **(ii) r^2_{QTL}:** proportion of the trait variance explained by the *DGAT1 K232A* polymorphism. **(iii) p-value $_{QTL}$:** statistical significance of the *DGAT1 K232A* effect. **(iv) $r^2_{polygenic}$:** proportion of the trait variance explained by the random, polygenic effect in the mixed model. **(v) r^2_{error}:** proportion of the trait variance unexplained by the model.

The two previous analyses examined the effect of the *DGAT1* polymorphism on estimated breeding values. By definition, this phenotype will only account for the additive component of the *DGAT1* effect, and justifies the use of a regression on the number of *K* alleles in the mixed model. To evaluate the dominance relationship between the *DGAT1* alleles, the effect of the *K232A* genotype on the lactation values (first yield deviations (Van Raden and Wiggans, 1991)) of the cows composing the New Zealand daughter design were analysed. This was achieved by using a mixed model including (i) a fixed effect corresponding to the *K232A* genotype, and (ii) a random polygenic component accounting for all known pedigree relationships ("animal model"). Very significant effects of *K232A* genotype on all examined yield and composition traits were found in this population as well (Table 3, below), accounting for between 1% (protein yield) and 31% (fat percentage) of the trait variance. The observed dominance deviations, *d*, corresponding to the difference between the genotypic value of the *KA*

genotype and the midpoint between the *AA* and *KK* genotypic values (Falconer and Mackey, 1996) are shown in Table 3 below. Genotypic values of the heterozygous genotype are systematically in between alternate homozygotes. None of the *d*-values proved to be significantly different from zero, indicating an absence of dominance. Average *K* to *A* QTL allele substitution effects, α (Falconer and Mackey, 1996), were computed from the estimates of *a*- and *d*-values, as well as the population frequencies of the *K* and *A* alleles (Table 3). The predicted substitution effects are in good agreement with those computed from the grand-daughter design: the *K* allele increases fat yield, fat % and protein %, while decreasing milk and protein yield. The absolute values of α estimated from the grand-daughter and daughter design are in perfect agreement for fat and protein %, while for the yield traits estimates are larger in the grand-daughter design when compared to the daughter design. The exact reasons for this are being explored. It could be due to the fact that the sire population in the grand-daughter design is not representative of the cow population in general, or to intrinsic differences between the Dutch and New-Zealand populations and/or environment.

Table 3:

Effect of the DGAT1 K232A mutation on cows' lactation values for milk yield and composition.

Trait	a	d	α	r^2_{QTL}	p-val $_{QTL}$	$r^2_{polygenic}$	r^2_{error}
Milk yield (Kgs)	-144 Kgs	-42 Kgs	-161 Kgs	0.03	1.05E-8	0.54	0.43
Fat yield (Kgs)	7.82 Kgs	-0.89 Kgs	7.46 Kgs	0.09	1.77E-20	0.46	0.45
Protein yield (Kgs)	-2.34 Kgs	-0.76 Kgs	-2.64 Kgs	0.01	4.35E-2	0.37	0.42
Fat %	0.41 %	0.03 %	0.42%	0.31	2.5E-108	0.49	0.20
Protein %	0.08%	0.03 %	0.08%	0.04	1.60E-20	0.72	0.24

(i) a: half the difference between the genotypic values of the *KK* and *AA* genotypes (Falconer and Mackey, 1996). **(ii) d:** dominance deviation (Falconer and Mackey, 1996): deviation of the *KA* genotypic value from the midpoint between the *AA* and *KK* genotypic values; none of these proved to be significantly different from zero. **(iii) α:** average *K* to *A* substitution effect, computed as "a + d(q-p)" (Falconer and Mackey, 1996), where *q* is the allelic frequency of *K* (=0.7) and *p* of *A* (=0.3) **(iv) r^2_{QTL}:** proportion of the trait variance explained by the *DGAT1 K232A* polymorphism. **(v) p-val $_{QTL}$:** statistical significance of the *DGAT1 K232A* effect. **(vi) $r^2_{polygenic}$:** proportion of the trait variance explained by the random, polygenic effect in the mixed model. **(vii) r^2_{error}:** proportion of the trait variance unexplained by the model.

Pedigree material and phenotypes. The pedigree material used for the association studies comprised a "grand-daughter design" (Weller et. al., 1990) counting 1,818 Holstein-Friesian bulls sampled in the Netherlands, as well as a "daughter-design" (Weller et. al., 1990) counting 529 Holstein-Friesian cows sampled in New Zealand. The phenotypes of the sires were "daughter yield deviations"(DYD: unregressed weighted averages of the daughter's lactation performances adjusted for systematic environmental effects and breeding values of the daughter's dams and expressed as deviations from the population mean (Van Raden and Wiggans, 1991)) obtained directly from CR-Delta (Arnhem – The Netherlands). The phenotypes of the cows were "lactation values" (first lactation yield deviations (YD), i.e. weighted average lactation performances expressed as deviations from the population mean, adjusted for management group, permanent environmental effects and herd-sire interaction effects (Van Raden and Wiggans, 1991)) obtained directly from Livestock Improvement Corporation (Hamilton – New Zealand).

Combined linkage and linkage disequilibrium analysis and association studies. The maximum likelihood procedure for combined linkage and linkage disequilibrium analysis is described in detail in Farnir, 2000. The association study in the grand-daughter design was performed using the following model:

$$y_i = \mu + \beta x_i + a_i + e_i$$

where y_i is the DYD of son i, μ is the overall population mean, β is a fixed regression coefficient estimating the *A* to *K* allele substitution effect, x_i is an indicator variable corresponding to the number of *K* alleles in the *K232A* genotype, a_i is a random polygenic component accounting for all known pedigree relationships ("animal model" Lynch and Walsh, 1997) and e_i is a random residual. The association study in the daughter design was performed using the model:

$$y_i = \mu + g_i + a_i + e_i$$

where y_i is the lactation value of cow i, g_i is a fixed effect corresponding to the *DGAT1* genotype (*KK*, *KA*, or *AA*), a_i is a random polygenic component accounting for all known pedigree relationships ("animal model" Lynch and Walsh, 1997) and e_i is a random residual. In both instances, maximum likelihood solutions for β, g_i ,a_i, e_i, σ^2_a, σ^2_e were obtained using the MTDFREML program (Boldman et al, 1997).

3. Genotype Testing and Analysis II

This summarises the genotype testing and subsequent analysis of Holstein-Friesian, Jersey and Ayrshire animals in a separate population from those presented in genotype testing and analysis I, above.

Progeny tested sires

Each year Livestock Improvement Corporation (New Zealand) progeny test some 200-300 bulls per year. This entails the bulls being genetically evaluated on the basis of 50-85 daughters per sire. The sires are evaluated for milk fat, milk protein, milk volume and 20 non-production traits. Semen has been retained from all progeny tested sires since the early 1970s. DNA was extracted from the semen and genotyped for the K232A DGAT1 polymorphism using the 7900 Taqman system (see Methods section below).

Statistical analysis was undertaken on this dataset using Restricted Maximum Likelihood (REML) and the average information algorithm (Johnson and Thompson, 1995). The linear model included the fixed effects of DGAT1 (3 classes; 0, 1 and 2 copies of the Q allele i.e. the K residue) and a covariate corresponding to the proportion of overseas genetics. The random effect was animal with a relationship matrix based on all known relationships. Daughter yield deviations (DYDs), weighted averages of a sire's daughter's lactation performances expressed as deviations from the population mean (van Raden and Wiggans 1991) were used as the phenotypic measurement. The phenotypes were weighted by a weighting factor based on the variance of the DYD for a son being:

$$\text{Var DYD} = \left[\frac{1+(n-1)\frac{1}{4}h^2}{n}\right]\sigma_p^2$$

where Var DYD is the variance of son's DYD; n is the number of daughters contributing to the DYD; h^2 is the heritability, which was taken as 0.35 for yield traits.

The dataset was analysed separately for the 3 major breeds; Holstein-Friesian, Jersey and Ayrshire.

Seventeen hundred and thirteen Holstein-Friesian sires were included in the analysis. The effect of the DGAT1 polymorphism was extremely significant for the three milk production traits (Table 4). With each additional Q allele the level of milk fat production increases by approximately 6 kg per lactation, milk protein production decreases by approximately 2.5 kg per lactation and milk volume decreases by approximately 125 litres per lactation.

Table 4:

Effect of the DGAT1 polymorphism on milk production in the Holstein-Friesian bull population (kilograms per lactation).

	Fat	Protein	Milk
qq	0	0	0
Qq	6.86	-2.13	-128
QQ	11.83	-4.80	-266
st. error	0.87	0.68	24

The effects for the Jersey and Ayrshire breeds were less significant than those of the Holstein-Friesian breed but were consistent in direction of effects.

Daughters for milk components

Data collection was integrated with LIC's herd testing service using a sample of 102 herds involved in LIC's Sire Proving Scheme (SPS) in 1995. In addition to milk volume from herd testing, the concentrations of fat, crude protein (total nitrogen), casein, whey and lactose were determined. The data was collected from over 3,000 cows born in 1996 and first calving in the 1998 spring season, these being predominantly the daughters of approximately 220 SPS bulls. The milk characteristics were measured at three herd tests on each cow, with each herd having a herd test in each of the Sept/Oct, Nov/Dec and Jan/Feb periods. The Milkoscan FT120, which employs Fourier transform infrared spectrophotometry with enhanced milk calibrations (Foss Electric Application Note Nos. 95, P/N 492280 and 102, P/N 578377), was used to determine the milk component concentrations.

Nine hundred and twelve daughters were genotyped for the DGAT1 polymorphism using the OLA system. Analysis was undertaken using SAS (Statistics, Version 5, 1985) fitting a general linear model. The model included sire and maternal grandsire as fixed effects, DGAT1 polymorphism (3 classes; 0, 1 and 2 copies of the Q allele), covariates including 16ths of Holstein-Friesian, Jersey, Ayrshire and other, proportion of overseas

genetics within the Holstein-Friesian, Jersey and Ayrshire breeds. Yield deviations that were pre-adjusted for herd, stage of lactation among other fixed effects were used (Johnson et al 2000).

The DGAT1 polymorphism is statistically significant for Lactose, casein, beta-casein and whey yield and also for casein and beta-casein percent as outlined in Table 5.

Table 5:

Effect of the DGAT1 polymorphism on milk components.

Trait	qq	Qq	QQ	p-value
Lactose yield*	48	23	0	<0.0001
Casein yield*	11.0	5.8	0	0.01
Casein %	-0.13	-0.06	0	<0.0001
Whey yield*	6.86	2.31	0	<0.0001
β-casein yield*	3.98	2.19	0	0.05
β -casein %	-0.43	-0.23	0	0.0001

* Units = g/day for lactose, casein and whey yield and g/litre for β-casein yield

Daughters for solid fat content

Six hundred and ninety-two daughters were phenotyped for solid fat content. Solid fat content of the milkfat is a characteristic which has a major influence on the functionality of milkfat products, and in particular has a significant effect on the hardness of butter (MacGibbon & McLennan, 1987). The solid fat content at 10°C (SFC 10) was used for comparison of the properties of the milkfat as it relates well to the sectility hardness measurement of butter, a major functional property. Thus the performance of milkfat products may be predicted from the characteristics of the milk produced. The solid fat content (SFC) of the extracted fat was determined by pulsed nuclear magnetic resonance (NMR) and expressed as percentage solid fat (MacGibbon & McLennan, 1987). As the milkfat was melted to remove any thermal history, prior to recrystallization under standard conditions, the SFC simply reflects the chemical composition of the milkfat.

The 692 daughters were a subset of the 912 daughters that were phenotyped and genotyped for the results presented in Table 2. The solid fat content measures were

collected over 2 lactations. Breeding values were calculated using an animal model similar to that of Johnson et al 2000.

The same statistical model was fitted for solid fat content as was for the milk component analysis. The DGAT1 polymorphism has a statistically significant effect (p-value <0.0001) on solid fat content, increasing it by approximately by 1% for each addition of the Q allele.

This effect was further confirmed in 50 daughters (predominantly Holstein-Friesian) that were farmed at one location and measured for SFC on the same day. The estimated effect for of the DGAT1 polymorphism on SFC was to increase it by approximately 2% per addition of each Q allele. This finding was significant at the five percent threshold level.

The genetic standard deviation for SFC is 2.25 (D Johnson personal communication) and thus the effect of DGAT1 is approximately 0.5 of a genetic standard deviation.

4. Relative Transcript Levels of the Splice Variant

Real time PCR experiments were conducted using reverse transcribed mRNA isolated from lactating bovine mammary gland(s) (see experimental methods). These experiments revealed that the alternatively spliced transcript as shown on Figure 2b, was approximately 100 fold less abundant than the full length transcript.

METHODS SECTION

In order to identify other polymorphisms within the bovine DGAT1 gene, DNA was isolated from sperm, PCR amplified and then using primers designed from the sequence shown in Figures 2a and 2b and/or the cDNA sequence (SEQ ID NO: 4) direct sequenced on an ABI 3100. The breeds examined were:

Ayrshire, Angler, Belgian Blue, Blond D'Aquitaine, Brown Swiss, Charolais, Red Devon, Devon, Dexter, Friesian, Guernsey, Belted Galloway, Gelbvieh, Hereford, Jersey, Limousin, Longhorn, Maine Anjou, MRI (Meuse-rhine-yssel), Murray Grey, Piedmontese, Romangola, Sahiwal, Santa Gertrudis, Scottish Highland, Shorthorn, South Devon, Sussex, Swedish Red, Simmental, Wagyu, Welsh Black, Angus, and Zebu.

All the polymorphisms discovered are listed in Table 1, above.

The majority of the primers are also listed in Figure 2b or contained in the cDNA sequence (SEQ ID NO: 4).

Experimental method for the OLA analysis of four SNP's in DGAT1

PCR amplification of the regions containing the polymorphisms

Protocol for the PCR amplification of exon VIII, intron XII and 3' UTR, the regions containing the four polymorphisms that were initially described in the DGAT1 gene.

Component	For 1 sample	Final concentration
HotStar Qiagen Buffer (10 x)	1.5 µl	0.7 µM
Primer 17F at 100µM	0.07 µl	0.7µM
18R at 10 µM	0.07 µl	0.5 µM
Primer 6F at 100µM	0.05µl	0.5 µM
AW 446985dn1 at 100µM	0.05µl	0.5 µM
Primer InsUp1	0.05µl	0.5 µM
14R2	0.05µl	10%
DMSO	1 µl	300 µM
dNTP 10mM	0.3 µl	0.1 U/µl
HotStarQiagen Taq (CatNr 203205:5U/µl)	0.2 µl	
H2O	1.66 µl	
DNA (5 ng/µl)	5 µl	
Total	10 µl	

Primer sequences are given in the following table as well as the genomic region targeted by them.

SNP targeted	Primer name	Primer sequence	5 ' base position
Exon VIII SNP (DG 1)	17F	CCTGAGCTTGCCTCTCCCACAGT	6579
	18R	CCAGGAGTCGCCGCAGCAGGAAG	7058
Exon XII SNPs	6F	CCGGCCATCCAGAACTCCATGAAG	7280
(DG 2 and DG3)	AW446985 dn1	TAGAACTCGCGGTCTCCAAAC	7605
	InsUp1	TGGCTGTCACTCATCATCGGGCA	8222
3'UTR SNP (DG4)	14R2	TTGCACAGCACTTTATTGACACA	8566

PCR amplification was performed on MJ PTC100 or PTCT200 cyclers using the following steps:

Step	Temperature	Time	Comment
1° Activation of the enzyme	94 °C	12 minutes	One times
2° Denaturation 3° Hybridisation 4° Elongation	92 °C 60°C 72°C	1 minute 1 minute 30 seconds 1 minute 30 seconds	Repeat step 2 to 4, 35 times
5° Inactivation of the enzyme	99°C	45 minutes	

Oligonucleotide Ligation Assay (OLA)

The oligonucleotides used in the OLA multiplex reaction are given in the table below. The detection of each mutation relies on the use of two fluorescent-labelled oligonucleotide (SNPx_FAM and SNPx_HEX) and one common 3' and 5' phosphorylated, non-labelled oligonucleotide (SNPx_2P)

Locus	Oligo	Sequence	5' base position	Number of spacer phosphoramidites	Size of the ligation product[a]
DG1	SNP1_FAM	AGC TTT GGC AGG TAA GGC	6813		
	SNP1_HEX	AGC TTT GGC AGG TAA GAA	6813		32
	SNP1_2P	GGC CAA CGG GGG AG	6831	0	
DG2	SNP2_FAM	GCT GGC GGT GAG TGA	7424		
	SNP2_HEX	GCT GGC GGT GAG TGG	7424		39
	SNP2_2P	CCT GCT GGG TGG GGA	7439	3	
DG3	SNP3_FAM	GCT GGG TGG GGA CGC	7442		
	SNP3_HEX	GCT GGG TGG GGA CGT	7442		29
	SNP3_P	GTG GGG GCG GGT GG	7457	0	
DG4	SNP4_FAM	TGC CCC AAC CTG GGT	8388		
	SNP4_HEX	TGC CCC AAC CTG GGC	8388		36
	SNP4_2P	GCA GCA GGA GGA GGC	8403	2	

[a] The size of the ligation products is the sum of the number of nucleotides of the two ligated oligonucleotides plus 3 bases equivalents per spacer phosphoramidites molecule, present at the 5' end of the common oligonucleotide.

For each SNP a mixture of the three oligonucleotides was prepared first, following the dilution guidelines in the table below.

SNP mixture	Oligonucleotide to mix	Quantity	Final concentration
DG1 (oligo. mixture)	SNP1_FAM 10 µM SNP1_HEX 10 µM SNP1_2P 10 µM H_2O	10 µl 20µl 20µl 50 µl	1 µM 2 µM 2 µM
DG2 (oligo. mixture)	SNP2_FAM 10 µM SNP2_HEX 10 µM SNP2_2P 10 µM H_2O	10 µl 20µl 20µl 50 µl	1 µM 2 µM 2 µM
DG3 (oligo. mixture)	SNP3_FAM 10 µM SNP3_HEX 10 µM SNP3_2P 10 µM H_2O	10 µl 20µl 20µl 50 µl	1 µM 2 µM 2 µM
DG4 (oligo. mixture)	SNP4_FAM 10 µM SNP4_HEX 10 µM SNP4_2P 10 µM H_2O	10 µl 30 µl 20 µl 40 µl	1 µM 3 µM 2 µM

The ligation reaction for one sample was performed as follow:

Component	Quantity persample
DG1 oligonucleotide mixture[a] (35, 70 and 70 nM) DG2 oligonucleotide mixture[a] (12.5, 25 and 25 nM) DG3 oligonucleotide mixture[a] (12.5, 25 and 25 nM) DG4 oligonucleotide mixture[a] (12.5, 37.5 and 25 nM) DMSO Incubation buffer of the Tsc DNA ligase (Roche, Cat Nr 1 939 807 or 1 939 815) Tsc DNA ligase H_2O Multiplex PCR (see above)	0.7 µl 0.25 µl 0.25 µl 0.25 µl 2 µl 2 µl 1µl 8.55 µl 5 µl
Total	20 µl

[a] The final concentration of the oligonucleotides in the ligation reaction is given between parenthesis (SNPx_FAM, SNPx_HEX and SNPx_2P respectively)

The sample was submitted to the following temperature cycling program in a MJ PTC100 or PTC 200 PCR machine.

Step	Temperature	Time	Comment
1° Initial denaturation step	98 °C	2 minutes	One times
2° Dénaturation 3° Hybridisation and ligation	94 °C 45°C	30 seconds 3 minutes	Repeat step 2 to 3, 30 times
5° Inactivation of the enzyme	99°C	45 minutes	

Following the LCR, 20 µl of H_2O was added to the ligation reaction. To 0.5 µl of the diluted ligation reaction, either 2 µl of loading buffer was added, or 2µl loading buffer containing TAMRA350 internal line size standard.

The loading buffer was composed as follows: 1 part of blue dextran (50mg/ml)/ EDTA (25mM) and 6 parts of formamide

The TAMRA350 containing loading buffer was composed as follows: 3 parts TAMRA350 (Applied Biosystems 401736 ; 8nM), 10 parts of Blue dextran (50 mg/ml)/EDTA (25 mM) and 60 parts of formamide.

TAMRA containing samples was placed alternately with TAMRA free samples when loaded onto the sequencing gel, in order to ease the identification of the lanes on the gel image.

The samples may require further dilution in order to avoid a too intense fluorescent signal on the sequencer. It is also very likely that from one primer batch to another, oligonucleotides concentrations will need adjustment.

The samples were denatured for 5 minutes at 95°C before loading. The samples were then loaded onto a 6% denaturing acrylamide gel on sequencer ABI 373 or a 4% gel on sequencer ABI 377.

In addition to the OLA assays referred to above, genotyping of the DGAT1 polymorphism was carried out by utilizing two different techniques for detection of PCR products.

Gel-based Genotyping Assay

Primer sequences 5' to 3', genomic sequence position in brackets:

DGAT1 21: GTAGCTTTGGCAGGTAAGAA (6811)
DGAT1 22: GGGGCGAAGAGGAAGTAGTA (6984)
DGAT1 23: TGGCCCTGATGGTCTACACC (6613)
DGAT1 24B: GGGCAGCTCCCCCGTTGGCCGC (6850)

The final reaction conditions were 1X Gold PCR buffer, 2.5mM $MgCl_2$ (Applied Biosystems), 200µM each dNTP (Roche), 600nM DGAT1 21 and DGAT1 22, 400nM DGAT1 23 and DGAT1 24B (Invitrogen), 10% dimethylsulphoxide (Sigma), 3µl DNA template and 2.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems) in a total volume of 50µl.

Cycling conditions were a 94°C initial denaturation for 5 minutes, then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 20 seconds followed by one extension cycle of 72°C for 2 minutes.

Primer positions around polymorphism (in bold) on genomic sequence from 6587 to 6986.

DGAT123 TGGC CCTGATGGTC TACACC

TGCCTCTCCC ACAGTGGGCT CCGTGCTGGC CCTGATGGTC TACACCATCC

TCTTCCTCAA GCTGTTCTCC TACCGGGACG TCAACCTCTG GTGCCGAGAG

CGCAGGGCTG GGGCCAAGGC CAAGGCTGGT GAGGGCTGCC TCGGGCTGGG

GCCACTGGGC TGCCACTTGC CTCGGGACCG GCAGGGGCTC GGCTCACCCC

DGAT1 21 GTAGCT TTGGCAGGTA AGAA

CGACCCGCCC CCTGCCGCTT GCTCGTAGCT TTGGCAGGTA AG**AA**GGCCAA

CGCCGGTT

CGGGGGAGCT GCCCAGCGCA CCGTGAGCTA CCCCGACAAC CTGACCTACC

GCCCCCTCGA CGGG DGAT1 24B

GCGGTGAGGA TCCTGCCGGG GGCTGGGGGG ACTGCCCGGC GGCCTGGCCT

GCTAGCCCCG CCCTCCCTTC CAGATCTCTA CTACTTCCTC TTCGCCCCCA

AT GATGAAGGAG AAGCGGGG DGAT1 22

The Q allele has polymorphic sequence AA and is detected by the DGAT1 21 + 22 primers, producing a band of 174bp. The q allele has polymorphic sequence GC and is detected by the DGAT123 + 24 primers, producing a band of 238bp.

The primers DGAT123 and DGAT122 also successfully PCR the DGAT1 gene producing a product of 372bp in all reactions. Therefore, a QQ homozygote would have bands at 372bp and 174bp, a qq homozygote would have bands at 372bp and 238bp and a Qq heterozygote would have all 3 bands at 372bp, 238bp and 174bp.

18µl of PCR product was separated on a 1.2% agarose TAE gel, stained with ethidium bromide and scored independently by two investigators on the basis of the number and size of bands present.

TaqMan Allelic Discrimination Genotyping Assay

Primer sequences 5' to 3', genomic sequence position in brackets:
DGAT1forAD: TTCTCCTACCGGGACGTCAA (6651)
ReverseNZ: CCGCGGTAGGTCAGGTTGTC (6890)

Probe sequences 5' to 3', genomic sequence position in brackets:
ForAA (FAM): CGTTGGCCTTCTTA (6838)
DGAT1ADGC (VIC): TTGGCCGCCTTACC (6836)
Both probes use MGB (minor groove binder) as a non-fluorescent quencher.

The final reaction conditions are 1x Universal PCR Mastermix (Applied Biosystems), 500nM each primer (Invitrogen), 70nM ForAA (FAM) probe, 300nM DGAT1ADGC (VIC) probe (Applied Biosystems) and 2µl of a 1/20 dilution of DNA template in a total volume of 10µl.
Cycling conditions were 50°C for 2 minutes, 95°C initial denaturation for 10 minutes, then 37 cycles of denaturation at 94°C for 15 seconds, annealing and extension 60°C for 1 minute.

Primer positions around polymorphism (in bold) on genomic sequence from 6587 to 6986.

TGCCTCTCCC ACAGTGGGCT CCGTGCTGGC CCTGATGGTC TACACCATCC

DGAT1forAD TTCTCC TACCGGGACG TCAA→
TCTTCCTCAA GCTGTTCTCC TACCGGGACG TCAACCTCTG GTGCCGAGAG

CGCAGGGCTG GGGCCAAGGC CAAGGCTGGT GAGGGCTGCC TCGGGCTGGG

GCCACTGGGC TGCCACTTGC CTCGGGACCG GCAGGGGCTC GGCTCACCCC

ForAA(FAM) A T TCTTCCGGTTGC

CGACCCGCCC CCTGCCGCTT GCTCGTAGCT TTGGCAGGTA AG**AA**GGCCAA

DGAT1ADGC (VIC) CCAT TCCGCCGGTT

CGGGGGAGCT GCCCAGCGCA CCGTGAGCTA CCCCGACAAC CTGACCTACC

← CTGTTG GACTGGATGG

GCGGTGAGGA TCCTGCCGGG GGCTGGGGGG ACTGCCCGGC GGCCTGGCCT CGCC ReverseNZ

GCTAGCCCCG CCCTCCCTTC CAGATCTCTA CTACTTCCTC TTCGCCCCCA

A 240bp product is produced in this reaction. When the Q allele (AA) is present the FAM-labelled probe binds and fluoresces at 518nm. When the q allele (GC) is present the VIC-labelled probe binds and fluoresces at 554nm. After cycling is complete, the plate is scanned on the ABI7900 Sequence Detection System, the fluorescence from each well detected, and a scattergraph is drawn. The scattergraph separates out into 3 clumps with Q homozygotes in the upper left hand corner, q homozygotes in the lower right hand corner and Qq heterozygotes in between. Each clump is circled and the software automatically determines the genotype for each sample. On each plate there are controls with 8 wells each of known Q homozygotes, q homozygotes, Qq heterzygotes and no template controls.

Splice Variant Gene Expression

To determine the relative gene expression of the splice variants created by insertion/deletion of 66bp around the polymorphic site by alternate exon usage, RNA was extracted from mammary tissue and reverse transcribed using oligodT primer using a first strand cDNA synthesis kit (Invitrogen). Real time PCR to determine relative quantities of each variant was then carried out.

Primer sequences 5' to 3', genomic sequence position in brackets:

DGAT1forRT66: TCTCCTACCGGGACGTCAAC (6652)

DGAT1revRT66: GAGATCGCGGTAGGTCAGGTT (6964)

DGAT1forRTless66: GCTGCTTTGGCAGATCTCTACTACTT (6711)

DGAT1revRTless66: AAGCGCTTTCGGATGCG (7038)

Probe sequences 5' to 3', genomic sequence position in brackets:
DGAT1with66 (FAM): CCGTGAGCTACCC (6857)

DGAT1less66 (VIC): CTTCGCCCCCACCCT (6976)

Both probes use MGB (minor groove binder) as a non-fluorescent quencher.

Final reaction conditions were 1X Universal PCR Mastermix (Applied Biosystems), 60nM each primer (Invitrogen), 60nM each probe (Applied Biosystems) and 1µl of template cDNA in a total volume of 10µl.

Cycling conditions were 50°C for 2 minutes, 95°C initial denaturation for 10 minutes, then 37 cycles of denaturation at 94°C for 15 seconds, annealing and extension 60°C for 1 minute.

Primer positions around 66bp insertion (in italics) on cDNA sequence. The start of the cDNA sequence is equivalent to position 6479 on the genomic sequence, with the last base of the cDNA equivalent to position 7428 of the genomic sequence.

CCGTGGCCTT TCTCCTCGAG TCTATCACTC CAGTGGGCTC CGTGCTGGCC

DGAT1forRT66 TCTCCT ACCGGGACGT
CTGATGGTCT ACACCATCCT CTTCCTCAAG CTGTTCTCCT ACCGGGACGT

CAAC→ DGAT1forRTless66 GCTGCTT
CAACCTCTGG TGCCGAGAGC GCAGGGCTGG GGCCAAGGCC AAGGCTGCTT

TGGCAG DGAT1with66(FAM) C CGTGAGCTAC
TGGCAG*GTAA GAAGGCCAAC GGGGGAGCTG CCCAGCGCAC CGTGAGCTAC*

CC ATCTCTAC TACTT →
*CCCGACAACC TGACCTACCG CG*ATCTCTAC TACTTCCTCT TCGCCCCCAC
← TTGG ACTGGATGGC GCTAGAGDGAT1revRT66 CT TCGCCCCCAC

CCTGTGCTAC GAGCTCAACT TCCCCCGCTC CCCCCGCATC CGAAAGCGCT
CCT DGAT1less66 (VIC) ← GCGTAG GCTTTCGCGA

TCCTGCTGCG GCGACTCCTG GAGATGCTGT TCCTCACCCA GCTCCAGGTG
A DGAT1revRTless66

GGGCTGATCC AGCAGTGGAT GGTCCCGGCC ATCCAGAACT CCATGAAGCC

CTTCAAGGAC ATGGACTACT CCCGCATCGT GGAGCGCCTC CTGAAGCTGG

This reaction detects the presence of the insertion splice variant by creating a 145bp product which binds the FAM probe only. The deletion splice variant is detected by a 92bp product that binds the VIC probe only.

The cDNA for each alternate splice variant was cloned into pGemT (Promega). A dilution series of the same, known amount, of each variant plasmid DNA was used to create a standard curve that established the linearity of the PCR reaction over a range of DNA concentrations. The threshold cycle number of the sample variants was converted back to a DNA amount by linear regression and the amounts of each variant present compared.

The presence of an alternate spice variant raises the possibility of an alternate function that is at this stage unknown.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, which may readily occur to a person skilled in the art, being possible without departing from the scope thereof as defined in the accompanying claims.

INDUSTRIAL APPLICATION

The present invention is directed to a method of genotyping bovine for improved milk production traits. In particular, such traits include increased milk volume and milk protein content and decreased milkfat content and solid fat content. It is anticipated that herds of bovine selected for such a trait will produce milk which will be more easily processed and such milk and products made therefrom may provide health benefits to consumers, as well as producing an increased milk yield.

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SEQUENCE LISTING

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GC corresponds to the q allele

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tcg cgg cct tcg atc cag ggc ggc agt ggg ccc gcg gca gcg gaa gag       96
Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
            20                  25                  30

gag gtg cgg gat gtg ggc gcc gga ggg gac gcg ccg gtc cgg gac aca      144
Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
        35                  40                  45
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cagcccccag cctcctggcc ctttggtgca cccacaaagc tccccccctcc cccaggagct 4906

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tgggtgggga gtctcagagc ttggcgtgag gtcccacagg gctgggcctg caggatggag 5746

gccactgtcc tgagctgcag gtgctggcag gagctggggt gggcgttctg gggccgtggc 5806

tgacagcgtt atgtccctct ctctctatcg cag atc tta agc aac gca cgg tta 5860
Ile Leu Ser Asn Ala Arg Leu

95 100

ttt cta gag aac ctc atc aa gtgagtgggc cccggcctgc cccagcccct 5910
Phe Leu Glu Asn Leu Ile Lys
105

gccacctcac ccctcgccta cacagaccct cacccacctg cgtctgcag g tat ggc 5966
Tyr Gly

atc ctg gtg gac ccc atc cag gtg gtg tct ctg ttc ctg aag gac ccc 6014
Ile Leu Val Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro
110 115 120 125

tac agc tgg cca gct ctg tgc ctg gtc att g gtgagctggg tgcccaggag 6065
Tyr Ser Trp Pro Ala Leu Cys Leu Val Ile
130 135

gcctcaggcc ggcggtgggt gggacagggc tgatctgggc ctgaacctgc cctgggttgc 6125

ttctgtcctc ag tg gcc aat atc ttt gcc gtg gct gcg ttc cag gtg gag 6175
Val Ala Asn Ile Phe Ala Val Ala Ala Phe Gln Val Glu
140 145

aag cgc ctg gcc gtg gtaagcagtg ccctcacgcc ctcccctgac ttgcctcaag 6230
Lys Arg Leu Ala Val
150

gtccttacca gtcgggctta gggcgggcca ccagctggtc ccactgtgct tcagggtttt 6290

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Gly

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Ala Leu Thr Glu Gln Ala Gly Leu Leu Leu His Gly Val Asn Leu Ala
155 160 165 170

acc att ctc tgc ttc cca gcg gcc gtg gcc ttt ctc ctc gag tct atc 6504
Thr Ile Leu Cys Phe Pro Ala Ala Val Ala Phe Leu Leu Glu Ser Ile
175 180 185

act cca g gtgggcccca cccccgcccc cgcccccgcc cacgctgtct cggccacggg 6561
Thr Pro

cagcgcgggg ggcgtggcct gagcttgcct ctcccacag tg ggc tcc gtg ctg 6614
Val Gly Ser Val Leu
190

gcc ctg atg gtc tac acc atc ctc ttc ctc aag ctg ttc tcc tac cgg 6662
Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr Arg
195 200 205

gac gtc aac ctc tgg tgc cga gag cgc agg gct ggg gcc aag gcc aag 6710
Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala Lys
210 215 220 225

gct g gtgagggctg cctcgggctg gggccactgg gctgccactt gcctcgggac 6764
Ala

cggcaggggc tcggctcacc cccgacccgc cccctgccgc ttgctcgtag ct ttg 6819
Ala Leu

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gca ggt aag aag gcc aac ggg gga gct gcc cag cgc acc gtg agc tac      6867
Ala Gly Lys Lys Ala Asn Gly Gly Ala Ala Gln Arg Thr Val Ser Tyr
    230                 235                 240

ccc gac aac ctg acc tac cgc g gtgaggatcc tgccgggggc tgggggact      6919
Pro Asp Asn Leu Thr Tyr Arg
245             250

gcccggcggc ctggcctgct agccccgccc tcccttccag at  ctc tac tac ttc     6973
                                            Asp Leu Tyr Tyr Phe
                                                        255

ctc ttc gcc ccc acc ctg tgc tac gag ctc aac ttc ccc cgc tcc ccc     7021
Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro
            260                 265                 270

cgc atc cga aag cgc ttc ctg ctg cgg cga ctc ctg gag atg              7063
Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Leu Leu Glu Met
        275                 280                 285

gtgaggcggg gcctcgcggg ccagggtggg cgggcctgcc ggcacccggc accggggctc   7123

agctcactgt ccgcttgctt ccttccccag ctg ttc ctc acc cag ctc cag gtg     7177
                                Leu Phe Leu Thr Gln Leu Gln Val
                                            290

ggg ctg atc cag cag gtacgtgccc gggggggggg gggggggggg gggggggact     7232
Gly Leu Ile Gln Gln
295

ctggggccgt tggggagctg actctgcgct ttttgcag tgg atg gtc ccg gcc atc    7288
                                        Trp Met Val Pro Ala Ile
                                        300                 305

cag aac tcc atg aag ccc ttc aag gtgagcaggc aggcctggca gggtgggttc     7342
Gln Asn Ser Met Lys Pro Phe Lys
                310

cggggtcagg gctgagggag ccagctgtgc cctgtgccca cag gac atg gac tac      7397
                                            Asp Met Asp Tyr
                                                315

tcc cgc atc gtg gag cgc ctc ctg aag ctg gcg gtgagtgacc tgctgggtgg    7450
Ser Arg Ile Val Glu Arg Leu Leu Lys Leu Ala
        320                 325

ggacgcgtgg gggcgggtgg ggctgttctg gcacctggca cccactcccc acag gtc      7507
                                                        Val

ccc aac cac ctc atc tgg ctc atc ttc ttc tac tgg ctc ttc cac tcc      7555
Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu Phe His Ser
330                 335                 340                 345

tgc ctg aac gcc gtg gct gag ctc atg cag ttt gga gac cgc gag ttc      7603
Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp Arg Glu Phe
                350                 355                 360

tac cgg gac tgg tg  gtgggtggcc ttgccggggc gggggtggtg ggggccccg      7657
Tyr Arg Asp Trp Trp
            365

ctggggctgg ggccggagcc cctgcccact ctgccccgcc cccgcag g aac tcc gag    7714
                                                    Asn Ser Glu
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tcc atc acc tac ttc tgg cag aac tgg aac atc cct gtt cac aag tgg     7762
Ser Ile Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp
370                 375                 380                 385

tgc atc ag  gtgggtgtgc gcctgggggc gggggggttgg ggggtgggac           7810
Cys Ile Arg

ggggtcgcgt ggcccgggcg cccagcccac tgccgcctcc cccgcag a cac ttc tac   7867
                                                    His Phe Tyr
                                                        390

aag ccc atg ctc cgg cgg ggc agc agc aag tgg gca gcc agg acg gca     7915
Lys Pro Met Leu Arg Arg Gly Ser Ser Lys Trp Ala Ala Arg Thr Ala
            395                 400                 405

gtg ttt ctg gcc tcc gcc ttc ttc cac gag gtcagtgcac tgagggcgcg       7965
Val Phe Leu Ala Ser Ala Phe Phe His Glu
        410                 415

ccctgcccct ggtgggggtg ggggtggggg tgggggctcg ctgacgcccc tctcccctca   8025

g tac ctg gtg agc atc ccc ctg cgc atg ttc cgc ctc tgg gcc ttc acc   8074
  Tyr Leu Val Ser Ile Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr
          420                 425                 430

ggc atg atg gcg cag gtgagcagcc ctggaccccc gctccgcccc gccccgcgag     8129
Gly Met Met Ala Gln
    435

cgcagaggct cactcccgtc ctgtgtcccc ag atc ccg ctg gcc tgg ata gtg     8182
                                  Ile Pro Leu Ala Trp Ile Val
                                      440                 445

ggc cgc ttc ttc cgc ggc aac tac ggc aac gcg gcc gtg tgg ctg tca     8230
Gly Arg Phe Phe Arg Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser
                450                 455                 460

ctc atc atc ggg cag ccg gtg gcc gtc ctg atg tac gtc cac gac tac     8278
Leu Ile Ile Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp Tyr
            465                 470                 475

tac gtg ctc aac cgt gag gcg ccg gca gcc ggc acc tgagcgcctc          8324
Tyr Val Leu Asn Arg Glu Ala Pro Ala Ala Gly Thr
        480                 485

caggctggcc ccctcgtggg tgttggactg ctttgccgcg ctgcctgcgg ctggactaga   8384

gcctgcccca acctgggcgc agcaggagga ggcctggctg gtggaagctg cctcctggcc   8444

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cctctactgg ctctgccgcc tgggttggaa accctgaggc tgtgccaggc aggtgtaccc 9224

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<210> 2

<211> 489

<212> PRT

<213> Bos taurus

<220>

<221> misc_feature

<222> (8315)..(8317)

<223> translation stop codon

<220>

<221> misc_feature

<222> (9434)..(9434)

<223> ambiguous nucleotide

<220>

<221> misc_feature

<222> (9496)..(9496)

<223> ambiguous nucleotide

<220>

<221> misc_feature

<222> (10402)..(10417)

<223> ambiguous nucleotides

<400> 2

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Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
            20                  25                  30

Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
        35                  40                  45

Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
    50                  55                  60

Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser
65                  70                  75                  80

Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser
                85                  90                  95

Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val
            100                 105                 110

Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp
        115                 120                 125

Pro Ala Leu Cys Leu Val Ile Val Ala Asn Ile Phe Ala Val Ala Ala
    130                 135                 140

Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Ala
145                 150                 155                 160

Gly Leu Leu Leu His Gly Val Asn Leu Ala Thr Ile Leu Cys Phe Pro
                165                 170                 175

Ala Ala Val Ala Phe Leu Leu Glu Ser Ile Thr Pro Val Gly Ser Val
            180                 185                 190

Leu Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr
        195                 200                 205

Arg Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala
    210                 215                 220

Lys Ala Ala Leu Ala Gly Lys Lys Ala Asn Gly Gly Ala Ala Gln Arg
225                 230                 235                 240

Thr Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe
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245 250 255

Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro
260 265 270

Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Leu Leu Glu Met Leu Phe
275 280 285

Leu Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met Val Pro Ala
290 295 300

Ile Gln Asn Ser Met Lys Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile
305 310 315 320

Val Glu Arg Leu Leu Lys Leu Ala Val Pro Asn His Leu Ile Trp Leu
325 330 335

Ile Phe Phe Tyr Trp Leu Phe His Ser Cys Leu Asn Ala Val Ala Glu
340 345 350

Leu Met Gln Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser
355 360 365

Glu Ser Ile Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys
370 375 380

Trp Cys Ile Arg His Phe Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser
385 390 395 400

Lys Trp Ala Ala Arg Thr Ala Val Phe Leu Ala Ser Ala Phe Phe His
405 410 415

Glu Tyr Leu Val Ser Ile Pro Leu Arg Met Phe Arg Leu Trp Ala Phe
420 425 430

Thr Gly Met Met Ala Gln Ile Pro Leu Ala Trp Ile Val Gly Arg Phe
435 440 445

Phe Arg Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser Leu Ile Ile
450 455 460

Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp Tyr Tyr Val Leu
465 470 475 480

Asn Arg Glu Ala Pro Ala Ala Gly Thr
485

<210> 3

<211> 31

<212> DNA

<213> Bos taurus

<220>

<221> misc_signal

<222> (29)..(31)

<223> bases 1 to 3 of the Kozak recognition sequence. See the genomic sequence from the start codon for bases 4 to 7 of the Kozak recognition sequence or the DGAT1 cDNA for the complete recognition sequence.

<400> 3
acttggccgc ggcggggtgc gaactaaggc c 31

<210> 4

<211> 1732

<212> DNA

<213> Bos taurus

<220>

<221> misc_feature

<222> (1)..(3)

<223> translation start codon

<220>

<221> misc_feature

<222> (1468)..(1470)

<223> translation stop codon

<220>

<221> variation

<222> (689)..(755)

<223> this sequence is deleted in the alternately spliced transcript

<220>

<221> variation

<222> (358)..(358)

<223> cytosine (C) to thyamine (T) substitution polymorphism

<220>

<221> variation

<222> (694)..(695)

<223> adenine (A)-adenine (A) to guanine(G)-cytosine (C) substitution p
olymorphism
AA corresponds to the Q allele
GC corresponds to the q allele

<220>

<221> primer_bind

<222> (616)..(635)

<223> Primer DgatforAD
TTCTCCTACCGGGACGTCAA

<220>

<221> primer_bind

<222> (743)..(767)

<223> Primer DgatrevAD
AAGTAGTAGAGATCGCGGTAGGTCA
reverse primer

<220>

<221> primer_bind

<222> (690)..(703)

<223> Primer ForAA (FAM)
CGTTGGCCTTCTTA
reverse primer

<220>

<221> primer_bind

<222> (688)..(709)

<223> Primer DgatADGC (VIC)
TTGGCCGCCTTACC
reverse primer

<220>

<221> primer_bind

<222> (617)..(636)

<223> Primer DgatforRT66
TCTCCTACCGGGACGTCAAC

<220>

<221> primer_bind

<222> (739)..(760)

<223> Primer DgatrevRT66
GAGATCGCGGTAGGTCAGGTT
reverse primer

<220>

<221> primer_bind

<222> (676)..(767)

<223> Primer DgatforRTless66
GCTGCTTTGGCAGATCTCTACTACTT
This primer selectively binds and amplifies a contigious sequence characteristic of the splice variant & generated by the deletion bases 689 to 755 of this sequence.

<220>

<221> primer_bind

<222> (817)..(833)

<223> Primer DgatrevRTless66
AAGCGCTTTCGGATGCG

<220>

<221> primer_bind

<222> (722)..(734)

<223> Primer Dgatwith66 (FAM)
CCGTGAGCTACCC

<220>

<221> primer_bind

<222> (771)..(785)

<223> Primer Dgatless66 (VIC)
CTTCGCCCCCACCCT

<400> 4
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atccagggcg gcagtgggcc cgcggcagcg gaagaggagg tgcgggatgt gggcgccgga 120
ggggacgcgc cggtccggga cacagacaag gacggagacg tagacgtggg cagcggccac 180
tgggacctga ggtgtcaccg cctgcaggat tccctgttca gttctgacag tggcttcagc 240
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tttctcctcg agtctatcac tccagtgggc tccgtgctgg ccctgatggt ctacaccatc 600
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ggggccaagg ccaaggctgc tttggcaggt aagaaggcca acgggggagc tgcccagcgc 720
accgtgagct accccgacaa cctgacctac cgcgatctct actacttcct cttcgccccc 780
accctgtgct acgagctcaa cttcccccgc tccccccgca tccgaaagcg cttcctgctg 840
cggcgactcc tggagatgct gttcctcacc cagctccagg tggggctgat ccagcagtgg 900
atggtcccgg ccatccagaa ctccatgaag cccttcaagg acatggacta ctcccgcatc 960
gtggagcgcc tcctgaagct ggcggtcccc aaccacctca tctggctcat cttcttctac 1020
tggctcttcc actcctgcct gaacgccgtg gctgagctca tgcagtttgg agaccgcgag 1080
ttctaccggg actggtggaa ctccgagtcc atcacctact tctggcagaa ctggaacatc 1140
cctgttcaca agtggtgcat cagacacttc tacaagccca tgctccggcg gggcagcagc 1200
aagtgggcag ccaggacggc agtgtttctg gcctccgcct tcttccacga gtacctggtg 1260
agcatccccc tgcgcatgtt ccgcctctgg gccttcaccg gcatgatggc gcagatcccg 1320
ctggcctgga tagtgggccg cttcttccgc ggcaactacg gcaacgcggc cgtgtggctg 1380
tcactcatca tcgggcagcc ggtggccgtc ctgatgtacg tccacgacta ctacgtgctc 1440
aaccgtgagg cgccggcagc cggcacctga gcgcctccag gctggccccc tcgtgggtgt 1500
tggactgctt tgccgcgctg cctgcggctg gactagagcc tgccccaacc tgggcgcagc 1560
aggaggaggc ctggctggtg gaagctgcct cctggcctcc accaggcctc tgcctgaagg 1620
gcttcctcct gccaggggag agcaggcccg acgcagttct ggcccctggg aggtgcccat 1680
gctctggaaa ccctacagat ctcgcccaag ggtctgaatg tgtcaataaa ga 1732

<210> 5

<211> 489

<212> PRT

<213> Bos taurus

<220>

<221> VARIANT

<222> (232)..(232)

<223> an amino acid substitution (K -> A) caused by a polymorphism at bases 7224-7225 of the genomic sequence (measured from the adenine residue of the translation start codon). Lysine (K) corresponds to the Q allele, alanine (A) corresponds to the q allele.

<400> 5

Met Gly Asp Arg Gly Gly Ala Gly Gly Ser Arg Arg Arg Arg Thr Gly
1 5 10 15

Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
20 25 30

Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
35 40 45

Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
50 55 60

Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser
65 70 75 80

Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser
85 90 95

Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val
100 105 110

Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp
115 120 125

Pro Ala Leu Cys Leu Val Ile Val Ala Asn Ile Phe Ala Val Ala Ala
130 135 140

Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Ala
145 150 155 160

Gly Leu Leu Leu His Gly Val Asn Leu Ala Thr Ile Leu Cys Phe Pro
165 170 175

Ala Ala Val Ala Phe Leu Leu Glu Ser Ile Thr Pro Val Gly Ser Val
180 185 190

Leu Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr
195 200 205

Arg Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala
210 215 220

Lys Ala Ala Leu Ala Gly Lys Lys Ala Asn Gly Gly Ala Ala Gln Arg
225 230 235 240

Thr Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe
245 250 255

Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro
260 265 270

Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Leu Leu Glu Met Leu Phe
275 280 285

Leu Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met Val Pro Ala
290 295 300

Ile Gln Asn Ser Met Lys Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile
305 310 315 320

Val Glu Arg Leu Leu Lys Leu Ala Val Pro Asn His Leu Ile Trp Leu
325 330 335

Ile Phe Phe Tyr Trp Leu Phe His Ser Cys Leu Asn Ala Val Ala Glu
340 345 350

Leu Met Gln Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser
355 360 365

Glu Ser Ile Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys
370 375 380

Trp Cys Ile Arg His Phe Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser
385 390 395 400

Lys Trp Ala Ala Arg Thr Ala Val Phe Leu Ala Ser Ala Phe Phe His
405 410 415

Glu Tyr Leu Val Ser Ile Pro Leu Arg Met Phe Arg Leu Trp Ala Phe
420 425 430

Thr Gly Met Met Ala Gln Ile Pro Leu Ala Trp Ile Val Gly Arg Phe
435 440 445

Phe Arg Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser Leu Ile Ile

450 455 460

Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp Tyr Tyr Val Leu
465 470 475 480

Asn Arg Glu Ala Pro Ala Ala Gly Thr
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<210> 6

<211> 467

<212> PRT

<213> Bos taurus

<400> 6

Met Gly Asp Arg Gly Gly Ala Gly Gly Ser Arg Arg Arg Arg Thr Gly
1 5 10 15

Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
20 25 30

Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
35 40 45

Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
50 55 60

Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser
65 70 75 80

Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser
85 90 95

Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val
100 105 110

Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp
115 120 125

Pro Ala Leu Cys Leu Val Ile Val Ala Asn Ile Phe Ala Val Ala Ala
130 135 140

Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Ala
145 150 155 160

Gly Leu Leu Leu His Gly Val Asn Leu Ala Thr Ile Leu Cys Phe Pro
165 170 175

```
Ala Ala Val Ala Phe Leu Leu Glu Ser Ile Thr Pro Val Gly Ser Val
            180                 185                 190

Leu Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr
        195                 200                 205

Arg Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala
    210                 215                 220

Lys Ala Ala Leu Ala Asp Leu Tyr Tyr Phe Leu Phe Ala Pro Thr Leu
225                 230                 235                 240

Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro Arg Ile Arg Lys Arg Phe
                245                 250                 255

Leu Leu Arg Arg Leu Leu Glu Met Leu Phe Leu Thr Gln Leu Gln Val
            260                 265                 270

Gly Leu Ile Gln Gln Trp Met Val Pro Ala Ile Gln Asn Ser Met Lys
        275                 280                 285

Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile Val Glu Arg Leu Leu Lys
    290                 295                 300

Leu Ala Val Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu
305                 310                 315                 320

Phe His Ser Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp
                325                 330                 335

Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser Glu Ser Ile Thr Tyr Phe
            340                 345                 350

Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp Cys Ile Arg His Phe
        355                 360                 365

Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser Lys Trp Ala Ala Arg Thr
    370                 375                 380

Ala Val Phe Leu Ala Ser Ala Phe Phe His Glu Tyr Leu Val Ser Ile
385                 390                 395                 400

Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr Gly Met Met Ala Gln
                405                 410                 415

Ile Pro Leu Ala Trp Ile Val Gly Arg Phe Phe Arg Gly Asn Tyr Gly
            420                 425                 430

Asn Ala Ala Val Trp Leu Ser Leu Ile Ile Gly Gln Pro Val Ala Val
        435                 440                 445
```

Leu Met Tyr Val His Asp Tyr Tyr Val Leu Asn Arg Glu Ala Pro Ala
450 455 460

Ala Gly Thr
465

<210> 7

<211> 12

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (7)..(7)

<223> thymidine (T) to guanine (G) substitution polymorphism

<400> 7
cagtgctagg gg 12

<210> 8

<211> 10

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> thymidine (T) to guanine (G) substitution polymorphism

<400> 8
gcattgcgct 10

<210> 9

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> thymidine (T) to cytosine (G) substitution polymorphism

<400> 9
taccctggga c 11

<210> 10

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> adenine (A) to guanine (G) substitution polymorphism

<220>

<221> variation

<222> (6)..(6)

<223> adenine (A) to guanine (G) substitution polymorphism

<400> 10
ctcttagcag c 11

<210> 11

<211> 10

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (5)..(5)

<223> guanine (G) to adenine (A) substitution polymorphism

<400> 11
acaggcaact 10

<210> 12

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> cytosine (C) to thyamine (T) substitution polymorphism

<400> 12
tgtctctgtt c 11

<210> 13

<211> 14

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (7)..(8)

<223> adenine (A)-adenine (A) to guanine(G)-cytosine (C) substitution p
olymorphism
AA corresponds to the Q allele
GC corresponds to the q allele

<400> 13
ggtaagaagg ccaa 14

<210> 14

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> guanine (G) to adenine (A) substitution polymorphism

<400> 14
gcggtgagga t 11

<210> 15

<211> 16

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(7)

<223> guanine (G)-guanine (G) to adenine (A)-cytosine (C) substitution polymorphism
GG-AC

<400> 15
gggggggggg gactct 16

<210> 16

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> adenine (A) to guanine (G) substitution polymorphism

<400> 16
gagtgacctg c 11

<210> 17

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> cytosine (C) to thymidine (T) substitution polymorphism

<400> 17
ggacgcgtgg g 11

<210> 18

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> guanine (G) to adenine (A) substitution polymorphism

<400> 18
ggtgggggtg g 11

<210> 19

<211> 11

<212> DNA

<213> Bos taurus

<220>

<221> variation

<222> (6)..(6)

<223> ctosine (C) to thymidine (T) substitution polymorphism

<400> 19

ctgggcgcag c 11

<210> 20

<211> 14

<212> DNA

<213> Bos taurus

<400> 20
cgttggcctt ctta 14

<210> 21

<211> 14

<212> DNA

<213> Bos taurus

<400> 21
ttggccgcct tacc 14

CLAIMS:

1. A method of determining genetic merit of a bovine with respect to milk composition and volume which comprises the step of determining the DGAT1 genotypic state of said bovine.

2. A method as claimed in claim 1, wherein the genotypic state is determined with respect to DNA, mRNA and/or protein obtained from said bovine by direct or indirect methods.

3. A method as claimed in claim 1, wherein the genotypic state is determined by the presence of at least one nucleotide difference from the nucleotide sequence encoding bovine DGAT1 (SEQ ID NO: 1) either by direct or indirect methods.

4. A method as claimed in claim 3, wherein the genotypic state is determined by the presence of one or more polymorphisms of SEQ ID NOs: 7 to 19 either by direct or indirect methods.

5. A method as claimed in claim 4, wherein the genotypic state is determined by detecting the presence of the K232A polymorphism (SEQ ID NO: 13), either by direct or indirect mehtods.

6. A method of selecting a bovine having a desired DGAT1 genotypic state comprising determining the genotypic state according to any one of claims 1 to 5 and selecting said bovine on the basis of said determination.

7. A bovine selected by the method of claim 6.

8. A method of identifying a bovine which possesses a genotype indicative of altered milk production traits, said method comprising:

obtaining a nucleic acid sample from said bovine and identifying a polymorphism selected from the group comprising SEQ ID NOs: 7 to 19 of the bovine DGAT1 gene,

wherein the presence of said polymorphism is associated with altered milk production traits.

9. A method as claimed in claim 8, wherein said altered milk production traits comprise an increase in milk volume and/or an increased protein to fat ratio of the milk composition or a decrease in milk volume and/or an increase in fat content of the milk composition.

10. A method as claimed in claim 8, wherein the polymorphism is K232A (SEQ ID NO: 13), wherein the presence or absence of an alanine is associated with the altered milk production traits as claimed in claim 9.

11. A method as claimed in claim 8, further comprising the step of amplifying said bovine DGAT1 gene sequence.

12. A method as claimed in claim 11, wherein primers selected from the group consisting of (SEQ ID NOs: 20 and 21) are used in said amplification.

13. A primer suitable for use in detecting a polymorphism selected from the group comprising SEQ ID NOs: 7 to 19 of the bovine DGAT1 gene, said primer consisting of a nucleotide sequence having about at least 12 contiguous bases of SEQ ID NOs. 1, 3 or 4.

14. A bovine identified by the method of any one of claims 8 to 12.

15. A bovine as claimed in claim 7 or 14, comprising a bull.

16. Semen produced by a bovine as claimed in claim 15.

17. A bovine as claimed in claim 7 or 14, comprising a cow.

18. Milk produced by a bovine as claimed in claim 17.

19. Milk as claimed in claim 18, comprising one or more altered characteristics selected from the group consisting of increased % protein; increased total protein content; increased protein to fat ratio; decreased total fat content; increased fat softness; increase unsaturated fat content; increased or decreased solid fat content; and increased or decreased total volume; when compared to milk produced by a non-genotyped bovine.

20. Milk as claimed in claim 18, which has a decreased solid fat content, and increased fat softness and is advantageous for use in dairy product processing plants.

21. A dairy product made from the milk as claimed in any one of claims 18 to 20.

22. A dairy product made from the milk as claimed in claim 20, wherein said product provides a beneficial health effect.

23. The use of the DGAT1 gene sequence (SEQ ID NOs: 1, 3 and 4) or a fragment or variant thereof, in the identification of one or more molecular DNA markers useful in genotyping and/or selecting a bovine according to the methods of any one of claims 1 to 6 and 8 to 12.

24. The use of one or more polymorphism sequences selected from the group consisting of SEQ ID NOs: 7 to 19 in a method of identification and selection of a bovine having at least one of said polymorphisms in its DGAT1 gene.

25. The use of a probe in the methods of genotyping according to any one of claims 1 to 6 and 8 to 12, wherein the probe is selected from any 5 or more contiguous nucleotides of the DGAT1 sequence of SEQ ID NOs: 1, 3 or 4, which is sufficiently complementary with said nucleic acid sequence so as to bind thereto under stringent conditions.

26. A kit for genotyping a bovine with respect to milk composition and volume associated with DGAT1, comprising a primer or probe as claimed in claim 13 or 25.

27. An isolated nucleic acid molecule having the sequence of SEQ ID NOs: 1 or 4 with or without one or more polymorphisms selected from the group consisting of SEQ ID Nos: 7 to 19, or a fragment or variant thereof.

28. An isolated nucleic acid molecule comprising a DNA molecule having in whole or in part the nucleotide sequence of SEQ ID NOs: 1, 3 or 4 or which varies from the sequence due to the degeneracy of the genetic code, or a nucleic acid strand capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

29. An isolated mRNA transcribed from DNA having a sequence which corresponds to a nucleic acid molecule as claimed in claim 27 or 28.

30. A recombinant cloning vector comprising the DNA molecule of claim 27 or 28.

31. A prokaryotic or eukaryotic cell containing the cloning vector of claim 30.

32. A transfected cell line which expresses a protein encoded by the nucleic acid molecule of claim 27 or 28.

33. A diagnostic kit useful in detecting a DNA molecule as claimed in claim 27 or 28, comprising a first and second primer for amplifying the DNA, the primers being complementary to nucleotide sequences of the DNA upstream and downstream, respectively, of a polymorphism in the portion of the DNA encoding DGAT1 which results in altered relative milk lipid and protein production and milk volume, wherein at least one of the nucleotide sequences is selected to be from a non-coding region of the DGAT1 gene.

34. A kit as claimed in claim 33, further comprising a third primer complementary to a polymorphism selected from the group consisting of of SEQ ID NO: 7 to 19 located on the DGAT1 gene.

35. A process for producing a protein encoded by a DNA molecule of claim 27 or 28, comprising the steps:

a) preparing a DNA fragment including a nucleotide sequence which encodes the protein;

b) incorporating the DNA fragment into an expression vector to obtain a recombinant DNA molecule which includes the DNA fragment and is capable of undergoing replication;

c) transforming a host cell with the recombinant DNA molecule to produce a transformant which can express the protein;

d) culturing the transformant to produce the protein; and

e) recovering the protein from resulting cultured mixture.

36. A purified protein having an amino acid sequence of SEQ ID NOs: 2, 5 or 6 or a fragment or variant thereof having biological activity of DGAT1.

37. An antibody raised against the protein as claimed in claim 36.

38. A method for the modulation of milk production and content in a bovine by modulating the activity of the DGAT1 protein.

39. A method as claimed in claim 38, comprising administering to the bovine an effective amount of a nucleic acid molecule substantially complementary to at least a portion of mRNA encoding the bovine DGAT1 protein and being of sufficient length to sufficiently reduce expression of said DGAT1.

40. An isolated antisense nucleic acid molecule for use in a method as claimed in claim 39.

41. A method of modulating milk production and/or milk content in a lactating bovine, including administering to the bovine an effective amount of a nucleic acid molecule having ribozyme activity and a nucleotide sequence substantially complementary to at least a portion of mRNA encoding a bovine DGAT1 and being of sufficient length to bind selectively thereto to sufficiently reduce expression of said DGAT1.

42. A method of inhibiting the activity of bovine DGAT1 in a lactating bovine so as to modulate milk production and/or milk solids content, comprising administering an effective amount of an antibody to the relevant DGAT1.

43. A method of modulating milk production and/or milk solids content by raising an autoantibody to a bovine DGAT1 in the bovine.

44. A transgenic animal having cells that contain a transgene, which transgene is introduced into the animal or an ancestor of the animal at a prenatal stage, wherein said transgene comprises a bovine cDNA comprising the nucleotide sequence of SEQ ID NO: 4 or an appropriate variant or subsequence thereof.

45. A transgenic non-human animal wherein said endogenous DGAT1 gene has been knocked out and replaced with a transgene.

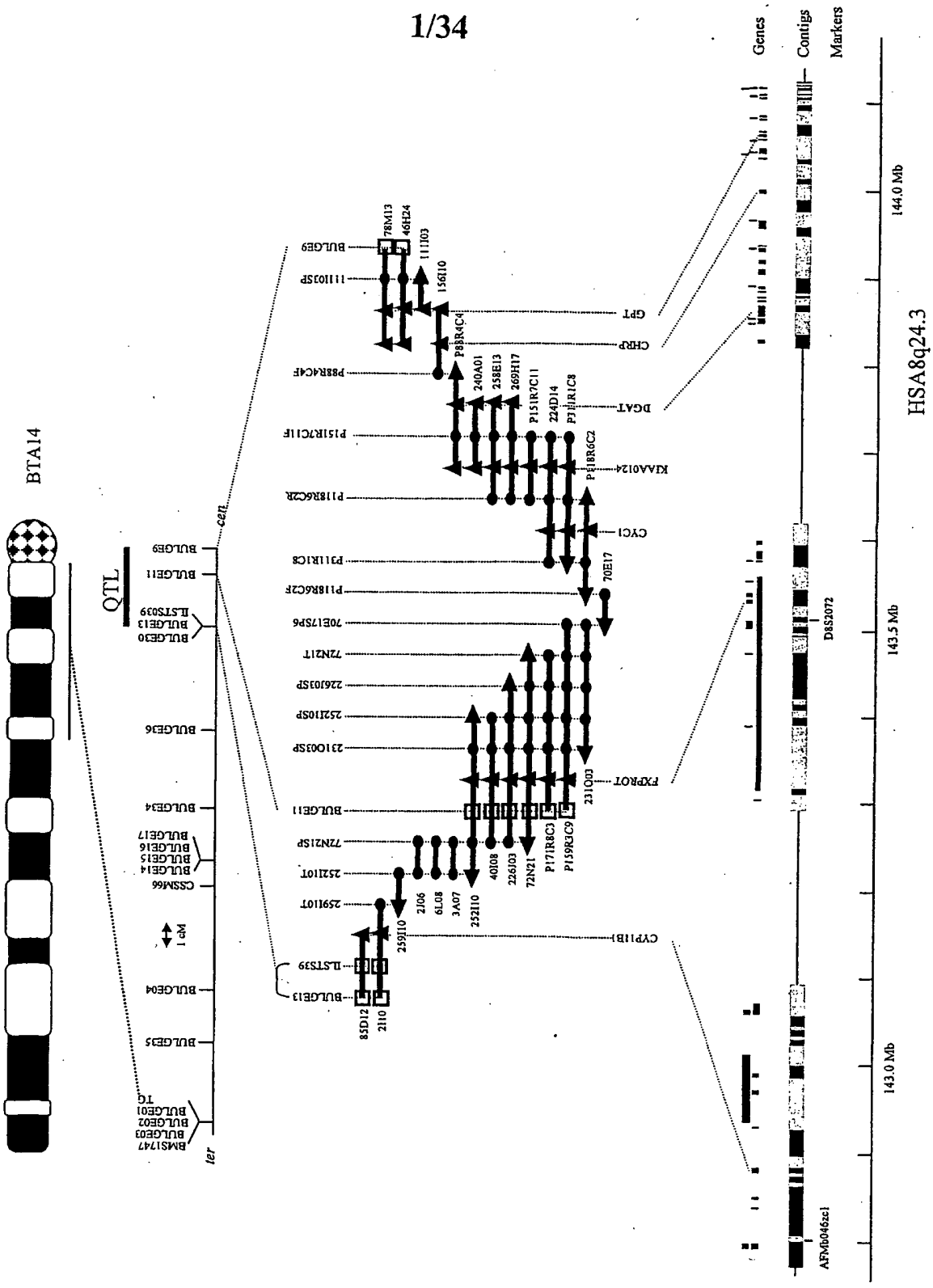
46. A transgenic non-human animal, wherein additional copies of the gene encoding DGAT1 are inserted as a transgene.

47. A transgenic non-human animal as claimed in claim 45 comprising a transgenic bovine having a genome lacking a gene encoding a protein having biological activity of DGAT1.

48. A transgenic non-human animal comprising a mouse having a genome containing a gene encoding a bovine protein having biological activity of DGAT1.

49. A transgenic bovine having a gene encoding a bovine protein having biological activity of bovine DGAT1 and heterologous nucleotide sequence antisense to the gene.

50. A transgenic bovine having a genome which includes additional copies of a gene encoding a protein having biological activity of DGAT1 or copies of a gene encoding a protein having biological activity of DGAT1 under control of a high expression promoter.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cen
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BULGE9
BULGE11
BULGE13
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ILSTS039
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BULGE15
BULGE14
CSSM66
1 cM
BULGE04
BULGE35
BULGE01
BULGE02
BULGE03
BMS1747
tq
Genes
Contigs
Markers
HSA8q24.3
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GPT
CHRP
DGAT
KIAA0124
CYC1
FXPROT
CYP11B1
78M13
46H24
111103
111103SP
156I10
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240A01
258E13
269H17
P151R7C11
224D14
P151R7C11F
P71R1C8
P118R6C2
P118R6C2R
P311R1C8
70E17
P118R6C2F
70E17SP6
72N21T
226J03SP
252I10SP
231O03SP
231O03
P171R8C3
P159R2C9
72N21SP
252I10T
259I10T
40I08
226J03
72N21
252I10
259I10
2J06
6L08
3A07
ILSTS39
BULGE13
85D12
2I10

FIGURE 1

<222> (29)..(31)

<223> bases 1 to 3 of the Kozak recognition sequence. See the genomic s
equence from the start codon for bases 4 to 7 of the Kozak recogn
ition sequence or the DGAT1 cDNA for the complete recognition seq
uence.

acttggccgc ggcggggtgc gaactaaggc c

FIGURE 2a

<210> 1

<211> 11771

<212> DNA

<213> Bos taurus

<220>

<221> CDS

<222> (1)..(191)

<223> Exon 1 CDS, determined by alignment with an amino acid sequence deduced from the cDNA

<220>

<221> misc_signal

<222> (1)..(4)

<223> these bases correspond to bases 4 to 7 of the Kozak recognition sequence. See DGAT1 cDNA for the complete recognition sequence.

<220>

<221> CDS

<222> (3809)..(3896)

<223> Exon 2, determined by alignment with an amino acid sequence deduced from the cDNA

<220>

<221> CDS

<222> (5840)..(5880)

<223> Exon 3, determined by alignment with an amino acid sequence deduced from the cDNA

FIGURE 2b

<220>

<221> CDS

<222> (5960)..(6045)

<223> Exon 4, determined by alignment with an amino acid sequence deduc
ed from the cDNA

<220>

<221> CDS

<222> (6138)..(6190)

<223> Exon 5, determined by alignment with an amino acid sequence deduc
ed from the cDNA

<220>

<221> CDS

<222> (6406)..(6511)

<223> Exon 6, determined by alignment with an amino acid sequence deduc
ed from the cDNA

<220>

<221> CDS

<222> (6601)..(6714)

<223> Exon 7, determined by alignment with an amino acid sequence deduc
ed from the cDNA

<220>

<221> CDS

<222> (6815)..(6889)

<223> Exon 8, determined by alignment with an amino acid sequence deduc
ed from the cDNA

FIGURE 2b continued

<220>

<221> variation

<222> (6824)..(6889)

<223> this sequence is deleted from the alternately spliced transcript.

<220>

<221> CDS

<222> (6960)..(7063)

<223> Exon 9, determined by alignment with an amino acid sequence deduc ed from the cDNA

<220>

<221> CDS

<222> (7154)..(7192)

<223> Exon 10, determined by alignment with an amino acid sequence dedu ced from the cDNA

<220>

<221> CDS

<222> (7271)..(7312)

<223> Exon 11, determined by alignment with an amino acid sequence dedu ced from the cDNA

<220>

<221> CDS

<222> (7386)..(7430)

<223> Exon 12, determined by alignment with an amino acid sequence ded uced from the cDNA

FIGURE 2b continued

<220>

<221> CDS

<222> (7505)..(7617)

<223> Exon 13, determined by alignment with an amino acid sequence dedu
ced from the cDNA

<220>

<221> CDS

<222> (7705)..(7770)

<223> Exon 14, determined by alignment with an amino acid sequence dedu
ced from the cDNA

<220>

<221> CDS

<222> (7858)..(7945)

<223> Exon 15, determined by alignment with an amino acid sequence dedu
ced from the cDNA

<220>

<221> CDS

<222> (8027)..(8089)

<223> Exon 16, determined by alignment with an amino acid sequence dedu
ced from the cDNA

<220>

<221> CDS

<222> (8162)..(8314)

<223> Exon 17 CDS, determined by alignment with an amino acid sequence
deduced from the cDNA

FIGURE 2b continued

<220>

<221> misc_feature

<222> (8315)..(8317)

<223> translation stop codon

<220>

<221> polyA_site

<222> (8572)..(8578)

<223>

<220>

<221> variation

<222> (626)..(626)

<223> thymidine (T) to guanine (G) substitution polymorphism

<220>

<221> variation

<222> (3512)..(3512)

<223> thymidine (T) to guanine (G) substitution polymorphism

<220>

<221> variation

<222> (4040)..(4040)

<223> thymidine (T) to cytosine (G) substitution polymorphism

<220>

<221> variation

<222> (4963)..(4963)

<223> adenine (A) to guanine (G) substitution polymorphism

FIGURE 2b continued

<220>

<221> variation

<222> (5003)..(5003)

<223> guanine (G) to adenine (A) substitution polymorphism

<220>

<221> variation

<222> (5997)..(5997)

<223> cytosine (C) to thyamine (T) substitution polymorphism

<220>

<221> variation

<222> (6829)..(6830)

<223> adenine (A)-adenine (A) to guanine(G)-cytosine (C) substitution p
olymorphism
AA corresponds to the Q allele
GC corresponds to the q allele

<220>

<221> variation

<222> (6892)..(6892)

<223> guanine (G) to adenine (A) substitution polymorphism

<220>

<221> variation

<222> (7224)..(7225)

<223> guanine (G)-guanine (G) to adenine (A)-cytosine (C) substitution
polymorphism
GG-AC

FIGURE 2b continued

<220>

<221> variation

<222> (7438)..(7438)

<223> adenine (A) to guanine (G) substitution polymorphism

<220>

<221> variation

<222> (7456)..(7456)

<223> cytosine (C) to thymidine (T) substitution polymorphism

<220>

<221> variation

<222> (7987)..(7987)

<223> guanine (G) to adenine (A) substitution polymorphism

<220>

<221> variation

<222> (8402)..(8402)

<223> ctosine (C) to thymidine (T) substitution polymorphism

<220>

<221> misc_feature

<222> (9434)..(9434)

<223> ambiguous nucleotide

<220>

<221> misc_feature

<222> (9496)..(9496)

<223> ambiguous nucleotide

FIGURE 2b continued

<220>

<221> misc_feature

<222> (10402)..(10417)

<223> ambiguous nucleotides

<220>

<221> primer_bind

<222> (6579)..(6601)

<223> Primer 17F
CCTGAGCTTGCCTCTCCCACAGT

<220>

<221> primer_bind

<222> (7036)..(7058)

<223> Primer 18R
CCAGGAGTCGCCGCAGCAGGAAG
reverse primer

<220>

<221> primer_bind

<222> (7280)..(7303)

<223> Primer 6F
CCGGCCATCCAGAACTCCATGAAG

<220>

<221> primer_bind

<222> (7585)..(7605)

<223> Primer AW446985 dn1
TAGAACTCGCGGTCTCCAAAC
reverse primer

FIGURE 2b continued

<220>

<221> primer_bind

<222> (8222)..(8244)

<223> Primer InsUp1
TGGCTGTCACTCATCATCGGGCA

<220>

<221> primer_bind

<222> (8566)..(8589)

<223> Primer 14R2
TTGCACAGCACTTTATTGACACA

<220>

<221> primer_bind

<222> (6813)..(6830)

<223> Primer SNP1_FAM
AGC TTT GGC AGG TAA GGC

<220>

<221> primer_bind

<222> (6813)..(6830)

<223> Primer SNP1_HEX
AGC TTT GGC AGG TAA GAA

<220>

<221> primer_bind

<222> (6831)..(6844)

<223> Primer SNP1_2P
GGC CAA CGG GGG AG

FIGURE 2b continued

<220>

<221> primer_bind

<222> (7424)..(7438)

<223> Primer SNP2_FAM
GCT GGC GGT GAG TGA

<220>

<221> primer_bind

<222> (7424)..(7438)

<223> Primer SNP2_HEX
GCT GGC GGT GAG TGG

<220>

<221> primer_bind

<222> (7439)..(7453)

<223> Primer SNP2_2P
CCT GCT GGG TGG GGA

<220>

<221> primer_bind

<222> (7442)..(7456)

<223> Primer SNP3_FAM
GCT GGG TGG GGA CGC

<220>

<221> primer_bind

<222> (7442)..(7456)

<223> Primer SNP3_HEX
GCT GGG TGG GGA CGT

FIGURE 2b continued

<220>

<221> primer_bind

<222> (7457)..(7470)

<223> Primer SNP3_P
GTG GGG GCG GGT GG

<220>

<221> primer_bind

<222> (8388)..(8402)

<223> Primer SNP4_FAM
TGC CCC AAC CTG GGT

<220>

<221> primer_bind

<222> (8388)..(8402)

<223> Primer SNP4_HEX
TGC CCC AAC CTG GGC

<220>

<221> primer_bind

<222> (8403)..(8417)

<223> Primer SNP4_2P
GCA GCA GGA GGA GGC

<220>

<221> primer_bind

<222> (6811)..(6830)

<223> Primer Dgat 21
GTAGCTTTGGCAGGTAAGAA

FIGURE 2b continued

<220>

<221> primer_bind

<222> (6965)..(6984)

<223> Primer Dgat 22
GGGGCGAAGAGGAAGTAGTA
reverse primer

<220>

<221> primer_bind

<222> (6613)..(6632)

<223> Primer Dgat 23
TGGCCCTGATGGTCTACACC

<220>

<221> primer_bind

<222> (6829)..(6850)

<223> Primer Dgat 24B
GGGCAGCTCCCCCGTTGGCCGC
reverse primer

<220>

<221> primer_bind

<222> (6651)..(6670)

<223> Primer DgatforAD
TTCTCCTACCGGGACGTCAA

<220>

<221> primer_bind

<222> (6871)..(6890)

<223> Primer ReverseNZ
CCGCGGTAGGTCAGGTTGTC
reverse primer

FIGURE 2b continued

<220>

<221> primer_bind

<222> (6825)..(6838)

<223> Probe ForAA (FAM)
CGTTGGCCTTCTTA

<220>

<221> primer_bind

<222> (6823)..(6836)

<223> Probe DgatADGC (VIC)
TTGGCCGCCTTACC

<220>

<221> primer_bind

<222> (6651)..(6670)

<223> Primer DgatforAD
TTCTCCTACCGGGACGTCAA

<220>

<221> primer_bind

<222> (6878)..(6972)

<223> Primer DgatrevAD
AAGTAGTAGAGATCGCGGTAGGTCA
reverse primer

<220>

<221> primer_bind

<222> (6825)..(6838)

<223> Probe ForAA (FAM)
CGTTGGCCTTCTTA

FIGURE 2b continued

<220>

<221> primer_bind

<222> (6823)..(6836)

<223> Probe DgatADGC (VIC)
TTGGCCGCCTTACC

<220>

<221> primer_bind

<222> (6652)..(6671)

<223> Primer DgatforRT66
TCTCCTACCGGGACGTCAAC

<220>

<221> primer_bind

<222> (6874)..(6964)

<223> Primer DgatrevRT66
GAGATCGCGGTAGGTCAGGTT
reverse primer

<220>

<221> primer_bind

<222> (6711)..(6972)

<223> Primer DgatforRTless66, GCTGCTTTGGCAGATCTCTACTACTT
This primer was designed to selectively bind and amplify the cDNA splice variant. The corresponding binding site in this genomic sequence comprises bases 6711 to 6715, 6815 to 6823 and 6960 to 6972.

<220>

<221> primer_bind

<222> (7022)..(7038)

<223> Primer DgatrevRTless66
AAGCGCTTTCGGATGCG
reverse primer

FIGURE 2b continued

<220>

<221> primer_bind

<222> (6857)..(6870)

<223> Probe Dgatwith66 (FAM)
CCGTGAGCTACCC

<220>

<221> primer_bind

<222> (6976)..(6990)

<223> Probe Dgatless66 (VIC)
CTTCGCCCCCACCCT

<400> 1

```
atg ggc gac cgc ggc ggc gcg ggc ggc tcc cgg cgc cgg agg acg ggg       48
Met Gly Asp Arg Gly Gly Ala Gly Gly Ser Arg Arg Arg Arg Thr Gly
1               5                   10                  15

tcg cgg cct tcg atc cag ggc ggc agt ggg ccc gcg gca gcg gaa gag       96
Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
            20                  25                  30

gag gtg cgg gat gtg ggc gcc gga ggg gac gcg ccg gtc cgg gac aca      144
Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
        35                  40                  45

gac aag gac gga gac gta gac gtg ggc agc ggc cac tgg gac ctg ag      191
Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
    50                  55                  60

gtagcggtgc gcgtgacccc taacctttga cccctgatac ggggcccctg cgacccaacc    251

tggtggccca ggcctgtcgg cggcagctcg ggctcgagtc cgagagtctg gcgcctggac    311

cttggtgcac agctgtgccc ctcgggcctc cacggggaaa cttagcggga ggttgggggc    371

ggagggtctc ctgcccggaa cacccaggta cgggggccga ggggagggca gcggctcaac    431

ttctagacgc cctcccctctg ccttcctttg gtgggttctg aagctttccc agggtgagcc    491

cactacgcac agtgtcctct acctggaagg agatacaggg gtccttcctg agggctatga    551

ggggtgcctt gtgggttgat aaagctcccg ggggaggagg gtggaccggc ggagaacaga    611

ggcaggggca gtgctagggg atttctcatc cctcgcagac cctccagaga atggtcttca    671

caaaggtccc tcatccgtca cccggcgatt gactggccta ggatcctgct tattaccagc    731

acaaatggct gctctagggt caaagtgggt cctgtaatgg gaccctcacc cctggttggg    791
```

FIGURE 2b continued

gtacagggga ggagttggaa gtgcgcacac ccacaggtgg gcgccctgct tagctgaagg 851

actgatggga aggagttggg ggagcaagct gcggctgaaa gggaggatct gacccacgtg 911

ggcatcagct aagtcctgct ggctgcctcc aggcgttccc tttgccatcc tccacgcccc 971

tccccgggg cctgaccttc atcctggtca agggctctca ggggctctgg ttttgggatc 1031

agctccagag ctagaggtta tcaaggagga agtgggcaac aggtcagtca gcaaggattt 1091

gctatcttca ctgggtgctg tggggagggg agggacaagg gcagttgggg tgcaggcact 1151

gtccctgccc ttgggggggca cacagttcac ctgagagata agatagccgc agccctgaag 1211

agtgagagca aaggtcaggc acagagttca ggatgacacc aggggagggt ggctctgtga 1271

ggggcactgg cttcctacag gccccaggtg gtcctgaggg ggcggctgca aaggccagga 1331

ggcccacagg cccctctgcc cactcctggg gaactggatt tggggtcact ttgtatgagg 1391

tgggggcggg taccagcttt gggccaagct gtcaccctgg atgggccatc acttgcctgc 1451

tctgtatagg ccagatggcc agaagctgct cctgtcctgt tgatggccca tcctcgaggt 1511

ctggaccctc gggaagagga gcagttggtg gcagggatgg gccaccggag accctcctga 1571

cctccaggac acgcagctgt gtgtgcctgt ccccaagcca catgccacat ggctaggggc 1631

ctcctggggc agggctgggc attggtctgg ctactcttgg tatcgcctat ggcttccctg 1691

cctcccagtc atcatcctcc cacctctgcc tccctgcctg ttcctctctt tctcctcagg 1751

cccttccgga catttcctgc tcacctaggt ctgggcaggc ggggtcaggt gccgggtgtg 1811

agctcactcc ttccggcagc aaggtgtagc tatgtgccgg aaggaaggcc gctgctgttg 1871

cctcgcctct gagtgcatcc cttccaggtc ctccacactc ccctgtgccc cgacacctgg 1931

tgcgtccttc agccattggt tcatgtgtcc tccaggcaca gctttctagt ccagagcctc 1991

taggctgggt gcaggaagtg ctgaggaagt ggcagccggg aggcgagctg gcaccctgtc 2051

cctccttgtt ctgtccgtcc ctgcccctgg accgtatggc cccgcatgtg tgatccccac 2111

ttggggctgt gcctctgggc aagttgggaa gcttggtgag cctcattttc atgtgcccgc 2171

ctcccagtac tgatgtgcag gttgaatgag gtgccaactg taatgagttg gaatggccct 2231

gctggctggg tgggactggg gagcaggtgg gggccgctgg ggggcacaga ggcacaccca 2291

gtgcctcagt cagggagagg gtgacagaga agctctgggt gaggccccac ctccactctg 2351

gccatggctg ctgccctttg gtccactgca gtgaactgtg ccatggggct ggacctctgt 2411

ggggattggt gggcagtggg ctttcttccc gcttggggcc tctgacctct gggggcaggg 2471

cgctgcccgg gtgggacagt cggaaggctg gtagagggac ctgaggggtc tgtgtggtgg 2531

ctgggggcag gcctcaggaa tttgacagca gggatctgga aaagctttaa taacattatt 2591

FIGURE 2b continued

```
tgttgtcagg attgggaaat gctcccctcc cccctccccc tctttcatct tagagactgc   2651

tgcacatctg gtcagtgtgg tcttcttggt ggcccccaag gtggcagggg tcacactgtt   2711

atgaaaccgt cccctgggta tgtggtgcag acatgcacat gcagatggtg attggcaggt   2771

tgtagcatga ggtggctttg ggacggttcc agtgacagtg agtgggctgg atctgggggg   2831

ttctgggcag gtccatcaag cggatacccc cacagactgt cctcttggga tagttgggcc   2891

tgggagccct gcttgccttg ccaaaaggca ggcgcagagt catgaagaag agggcttggg   2951

ggctcagagc cccactgtgt gtgcagccca gggtggacct ggaggaggtg cgtgggcagg   3011

ctgggccggc cggggcgggg ggtgggggggg cctggtgtga aagggaccca gggccagact   3071

gtcagcgctg cctggctgag gatgctggca ccctgtcctc cccagccgtc tgtctcctgg   3131

gtgcagccat ctgagtgctg accccagccg cccctggagg ctggctgttc tcctgtgccc   3191

tattgctggg gacatgtgtc cacaggaggg aaagggaagc cccggcctct ccccttacaa   3251

aactggaggc cttgctcaat gccctggatg gcctcctggt ggcagggtgg ttggtgggag   3311

gtggggctgc tgcttagaac ccgccagcgg gcctgggcct gggctgagct gcacccctcc   3371

acctctgcct ccagctgagg gttggcttcc atctccacca ggcccagcac tgggcacagg   3431

gctctcagag gcaggctctg aaagtcccct gctggcttct gcagtggact ccaggcgccg   3491

agcccccagg gggctcgcat tgcgctcacc ctgcgaagcc acgtgaaggc tgggtcctcc   3551

cctccggaag ggccaaatgc agggcatggg tggtttgaat ggtggccccc gggctccccg   3611

gagggaccag ctgctgtgag ggccgccccc tccccacttc cgtcttgcat caccagctcc   3671

tgtggcactc cccacgcccc gtcccccagt gggagcggca ggcccccggt ggctctgccc   3731

gcggaggggg atgtgtgggc ggcggggtgg ccttgctgcc agatgctctg ccccgagtgt   3791

ccgtctccgc tctccag g tgt cac cgc ctg cag gat tcc ctg ttc agt tct     3842
                    Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser
                    65                  70                  75

gac agt ggc ttc agc aac tac cgt ggc atc ctg aat tgg tgt gtg gtg     3890
Asp Ser Gly Phe Ser Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val
                80                  85                  90

atg ctg gtacgtagag tgacaccttg gagcaagggt cctgacggcc ggggggccat      3946
Met Leu


gggctcttct ccaggggtag gtgtctgtac ttgtgtagct gtggtgaatg gagctctgtg   4006

ctggcggtgg gggtccctgg agcagccgta ccctgggacc ctaccgggag catgctcatg   4066

ccgtccctgc tgaatcccag gagatgcctg cagagggcag cctgggagcc tctgagctgg   4126

ggtctgcgcc ccaggggggca ctggagtctc cccaggggggc gagagagagt aggcagggat   4186
```

FIGURE 2b continued

```
ggtctggtgg ccctgggtgg gggatggctg ctccgtgggc ccaggccctc cctggcagca   4246

caggtgagtg gtcttggggg tccacgtaga acttcctctt ctgttccaaa ttgccctcat   4306

gggtgcggca tgcctgggtg aacctggggg agcagggtga ggacatgctt ctcagcccag   4366

cccacagctc caggccacac tctgcaggac tctggcccct ccctcagccc tggagggagc   4426

aggactggag tcctgtgtcc gccttgctct gacctggccg aggccactgc tgtggggccc   4486

cagcaggcct gcccagcaga aggtggagtg cagggacccc aggggcagcc ttcagggtgg   4546

ggcagggtga ggcccgactg ggcccagccc caccgctcag tgctgatgtg gcgcgaggcc   4606

ttcgcccctc cagctgacgt gtctgcctgc cctgggtgtg gctccagagg ctgcctgtgt   4666

accaggggcc cccacgcttc tgtttgtggt tctgggcagt cccctgggga gcggtggggg   4726

ctgtgtgcca gtccagaccc agtagtccac gcgtcctggt ctctggaggc cgtggctggt   4786

ccaggactgt ggcaaggtgg tcgtgcaggg caggccctca gcagcctgtc tgttctcctg   4846

cagcccccag cctcctggcc ctttggtgca cccacaaagc tccccctcc cccaggagct   4906

ggggccgcct gctgcgtcct ctcggcagcc tgggcttcca ggtggctggg cctcttagca   4966

gctccaactc ttgcctgtgg tgggctctca ggacaggcaa ctgccagtcg gcagacattg   5026

caggaccacg tgtgtcctgg taagctggct ggttaggtgt ttagctgggg gatggtgtgg   5086

caggtggccc ctgcatctct gagcctgtca cctcctcggg aagccttctg ggtgggggac   5146

tccacccatg tcgcctggag aagcatcact tttccacaga gccttctgca acccccgtgg   5206

ggcctgagcc tggggtgggg gaggtggtgg cccctgctcc tgcagaggcc agccaggcat   5266

ctggccccag gccactggca agagctcgtt gtgttggggg atctgtcctt tgctgctgct   5326

gcaggagcgg ccgaggcagg cgggggcgtg agtaggggtg gagacccagg cccagcttcc   5386

ccagcccctc aggaccggcc tgctctttcc caccacccca ccaagtgcgt gggcacaccc   5446

cgcctgtgag gatgggcccg gttggcaggg cggagccctg ggagggtggc agtgcgccgg   5506

gcaggcttgg acttcactgg ggcttggggt tgtcgctgtg gccaggggcg ctgacccgct   5566

tggtgggacg gacggccgct gggcagcagg tttcttctgc cacggtggca caggcacctg   5626

gggttgtggt tggctccagg cgggcggggg ctgcgtgccc ctgcgcaggc acataggccg   5686

tgggtgggga gtctcagagc ttggcgtgag gtcccacagg gctgggcctg caggatggag   5746

gccactgtcc tgagctgcag gtgctggcag gagctggggt gggcgttctg gggccgtggc   5806

tgacagcgtt atgtccctct ctctctatcg cag atc tta agc aac gca cgg tta     5860
                                    Ile Leu Ser Asn Ala Arg Leu
                                        95                  100

ttt cta gag aac ctc atc aa  gtgagtgggc cccggcctgc cccagcccct         5910
Phe Leu Glu Asn Leu Ile Lys
                105
```

FIGURE 2b continued

```
gccacctcac ccctcgccta cacagaccct cacccacctg cgtctgcag g tat ggc      5966
                                                          Tyr Gly

atc ctg gtg gac ccc atc cag gtg gtg tct ctg ttc ctg aag gac ccc      6014
Ile Leu Val Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro
110                 115                 120                 125

tac agc tgg cca gct ctg tgc ctg gtc att g gtgagctggg tgcccaggag      6065
Tyr Ser Trp Pro Ala Leu Cys Leu Val Ile
                130                 135

gcctcaggcc ggcggtgggt gggacagggc tgatctgggc ctgaacctgc cctgggttgc    6125

ttctgtcctc ag tg  gcc aat atc ttt gcc gtg gct gcg ttc cag gtg gag      6175
              Val Ala Asn Ile Phe Ala Val Ala Ala Phe Gln Val Glu
                            140                 145

aag cgc ctg gcc gtg gtaagcagtg ccctcacgcc ctcccctgac ttgcctcaag      6230
Lys Arg Leu Ala Val
    150

gtccttacca gtcgggctta gggcgggcca ccagctggtc ccactgtgct tcagggtttt    6290

gggcctttcg tggccttcct gagaggggct gcacctcagg cctggtggct cttcctcagg    6350

gaggtcctct gaccagggag gggggtccct ggctgacgct ctgctcccac cccag gga     6408
                                                             Gly

gct ctg acg gag cag gcg ggg ctg ctg ctg cac ggg gtc aac ctg gcc      6456
Ala Leu Thr Glu Gln Ala Gly Leu Leu Leu His Gly Val Asn Leu Ala
155                 160                 165                 170

acc att ctc tgc ttc cca gcg gcc gtg gcc ttt ctc ctc gag tct atc      6504
Thr Ile Leu Cys Phe Pro Ala Ala Val Ala Phe Leu Leu Glu Ser Ile
                175                 180                 185

act cca g gtgggcccca cccccgcccc cgcccccgcc cacgctgtct cggccacggg     6561
Thr Pro

cagcgcgggg ggcgtggcct gagcttgcct ctcccacag tg  ggc tcc gtg ctg       6614
                                          Val Gly Ser Val Leu
                                              190

gcc ctg atg gtc tac acc atc ctc ttc ctc aag ctg ttc tcc tac cgg      6662
Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr Arg
    195                 200                 205

gac gtc aac ctc tgg tgc cga gag cgc agg gct ggg gcc aag gcc aag      6710
Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala Lys
210                 215                 220                 225

gct g gtgagggctg cctcgggctg gggccactgg gctgccactt gcctcgggac        6764
Ala

cggcaggggc tcggctcacc cccgacccgc cccctgccgc ttgctcgtag ct  ttg      6819
                                                        Ala Leu
```

FIGURE 2b continued

```
gca ggt aag aag gcc aac ggg gga gct gcc cag cgc acc gtg agc tac     6867
Ala Gly Lys Lys Ala Asn Gly Gly Ala Ala Gln Arg Thr Val Ser Tyr
    230                 235                 240

ccc gac aac ctg acc tac cgc g gtgaggatcc tgccgggggc tgggggact      6919
Pro Asp Asn Leu Thr Tyr Arg
245                 250

gcccggcggc ctggcctgct agccccgccc tcccttccag at  ctc tac tac ttc     6973
                                            Asp Leu Tyr Tyr Phe
                                                        255

ctc ttc gcc ccc acc ctg tgc tac gag ctc aac ttc ccc cgc tcc ccc     7021
Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro
            260                 265                 270

cgc atc cga aag cgc ttc ctg ctg cgg cga ctc ctg gag atg              7063
Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Leu Leu Glu Met
        275                 280                 285

gtgaggcggg gcctcgcggg ccagggtggg cgggcctgcc ggcacccggc accggggctc   7123

agctcactgt ccgcttgctt ccttccccag ctg ttc ctc acc cag ctc cag gtg     7177
                                Leu Phe Leu Thr Gln Leu Gln Val
                                            290

ggg ctg atc cag cag gtacgtgccc gggggggggg gggggggggg gggggggact     7232
Gly Leu Ile Gln Gln
295

ctggggccgt tggggagctg actctgcgct ttttgcag tgg atg gtc ccg gcc atc   7288
                                        Trp Met Val Pro Ala Ile
                                        300                 305

cag aac tcc atg aag ccc ttc aag gtgagcaggc aggcctggca gggtgggttc    7342
Gln Asn Ser Met Lys Pro Phe Lys
                310

cggggtcagg gctgagggag ccagctgtgc cctgtgccca cag gac atg gac tac     7397
                                                Asp Met Asp Tyr
                                                    315

tcc cgc atc gtg gag cgc ctc ctg aag ctg gcg gtgagtgacc tgctgggtgg   7450
Ser Arg Ile Val Glu Arg Leu Leu Lys Leu Ala
        320                 325

ggacgcgtgg gggcgggtgg ggctgttctg gcacctggca cccactcccc acag gtc     7507
                                                            Val


ccc aac cac ctc atc tgg ctc atc ttc ttc tac tgg ctc ttc cac tcc     7555
Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu Phe His Ser
330                 335                 340                 345

tgc ctg aac gcc gtg gct gag ctc atg cag ttt gga gac cgc gag ttc     7603
Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp Arg Glu Phe
                350                 355                 360
```

FIGURE 2b continued

```
  tac cgg gac tgg tg  gtgggtggcc ttgccggggc gggggtggtg ggggccccg     7657
Tyr Arg Asp Trp Trp
           365

ctggggctgg ggccggagcc cctgcccact ctgccccgcc cccgcag g aac tcc gag   7714
                                                      Asn Ser Glu


tcc atc acc tac ttc tgg cag aac tgg aac atc cct gtt cac aag tgg     7762
Ser Ile Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp
370                 375                 380                 385

tgc atc ag  gtgggtgtgc gcctgggggc gggggggttgg ggggtgggac           7810
Cys Ile Arg


ggggtcgcgt ggcccgggcg cccagcccac tgccgcctcc cccgcag a cac ttc tac   7867
                                                     His Phe Tyr
                                                         390

aag ccc atg ctc cgg cgg ggc agc agc aag tgg gca gcc agg acg gca     7915
Lys Pro Met Leu Arg Arg Gly Ser Ser Lys Trp Ala Ala Arg Thr Ala
           395                 400                 405

gtg ttt ctg gcc tcc gcc ttc ttc cac gag gtcagtgcac tgagggcgcg       7965
Val Phe Leu Ala Ser Ala Phe Phe His Glu
       410                 415

ccctgccccc ggtgggggtg ggggtggggg tgggggctcg ctgacgcccc tctcccctca   8025

g tac ctg gtg agc atc ccc ctg cgc atg ttc cgc ctc tgg gcc ttc acc   8074
  Tyr Leu Val Ser Ile Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr
         420                 425                 430

ggc atg atg gcg cag gtgagcagcc ctggaccccc gctccgcccc gccccgcgag     8129
Gly Met Met Ala Gln
    435

cgcagaggct cactcccgtc ctgtgtcccc ag atc ccg ctg gcc tgg ata gtg     8182
                                  Ile Pro Leu Ala Trp Ile Val
                                      440                 445

ggc cgc ttc ttc cgc ggc aac tac ggc aac gcg gcc gtg tgg ctg tca     8230
Gly Arg Phe Phe Arg Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser
               450                 455                 460

ctc atc atc ggg cag ccg gtg gcc gtc ctg atg tac gtc cac gac tac     8278
Leu Ile Ile Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp Tyr
           465                 470                 475

tac gtg ctc aac cgt gag gcg ccg gca gcc ggc acc tgagcgcctc          8324
Tyr Val Leu Asn Arg Glu Ala Pro Ala Ala Gly Thr
       480                 485

caggctggcc ccctcgtggg tgttggactg ctttgccgcg ctgcctgcgg ctggactaga   8384

gcctgcccca acctgggcgc agcaggagga ggcctggctg gtggaagctg cctcctggcc   8444

tccaccaggc ctctgcctga agggcttcct cctgccaggg gagagcaggc ccgacgcagt   8504

tctggcccct gggaggtgcc catgctctgg aaaccctaca gatctcgccc aagggtctga   8564
```

FIGURE 2b continued

atgtgtcaat aaagtgctgt gcacagtgag ctccctcagc ctccagggca cagggctggc 8624

aggaggggc ggccctccca cgtggggcca tgctgtggga aggaggcccc agcgcctgga 8684

gaggagctgg ggctgtggtg accctccctg cctcacaggg ctctgtggtc agacgtcttg 8744

ccctgcaagg tggagactcc atgctccaag gccccctgtg cctgaggtct gcacacaagt 8804

ggattcaact tgggtcaggc cagaggctaa ggtgtggaag aaggttgaga atcaggctga 8864

cttgaacggc agcaaagact ccaaggcaag gctgcagaag tctcaaaagc tatgcgcaca 8924

gtcccctgct ggggtgctca cctgggctgg gctctgggct gcttggacaa agcaggtggc 8984

ctggctcagc cctcaccgag ggccttcctt gggggcagag gttggcctga tgccaggggc 9044

tccccgtttt tccaggccct cagcaggtag ttgggtgtgg ccctcatgat accttggtcc 9104

cagagctccg ccactcaaaa agcttggcag tgaggcaagg gcaaccccgg gctgttcccc 9164

cctctactgg ctctgccgcc tgggttggaa accctgaggc tgtgccaggc aggtgtaccc 9224

tgacagccag ccatggccca gtaagatggg tgcccgaggt ggtacctggg cagcggaccc 9284

agctgtgctg cccccgcccc aaccagaagc cgctctagcc catggtggtc gtctgggcga 9344

gacaggctgg ttggctaggc actgtttggt ctacagcagg tgtaggcagc gtctccctga 9404

cccctgcctt ctaggaagcc accaccctgn gccctactca tcagcaagga cagcgagcag 9464

ggctgagctg ggggtgcgtg ggctgctacg gnccccgcac cttcatcaca tgcacctctg 9524

caccccctgc tgcctgactc aagagtgggg ggggggggtcc tgtgcttcct tcattccaga 9584

cccacggtgc tgacccagtg cacccacctg gtccgctagt gctgacctgg ccacagggct 9644

cctgtgggcc cacgctgatc ccgccctggt cccttcataa agaactcttg agcacatgca 9704

gcccagggga gccaggaggc tccagtgtgc tgtgtccatc tgcctccctt cagccccttc 9764

cgagacactg cgcatcatgc ccccctccac ccccacccac actggcagga ggaacacaca 9824

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tccatgtata tatctgcata tctctatgga aggggaaagg gggactcgtg taaaaatcca 9944

aaatacaatt ctatgaacac ctgcatcctg gtcagtctga gtgtggccgt gaagcccagg 10004

tgagctgtgg ctcacagggc taggccctcg gtgctggccg ggggccactc cccacccccct 10064

ctcccccccct ccgccagcca ggggaccagg ctcctggaca ccaggcctgc ccaaggcctg 10124

ctctcctcct ggggcttcta cgagacagtg gggtccttgg ctttgggggg ttctgagccc 10184

gtcagcaggg agatggtggg gtcatctctt atatttcgtc tccctcggag aagtaggagc 10244

cctcccccag ctcgaagagc accggcaggt cgctgctccc cacgtccacg gagcccgggt 10304

ccaggagcag caggggctgg gcggtgtagt gcaccagctg cttccctagg ggtgcgactg 10364

FIGURE 2b continued

ggtcagggtg ccggtggggc cgggggcgg ggtggggnnn nnnnnnnnnn nnnccccccc 10424

ccccggcccc agccaccccg cctacgcacg ctggccaggc tgctgtccag gtcgggcagg 10484

ctcatgtcgg gcaccgtaac cgaggggctg aacagctgca gggaagaggg gcgggtcaga 10544

ctgccctgga agcggggcgg gctgggcgcg gggcgggcag ggttagactc acatccagca 10604

gggtgctggt gtccacgctg aagccatggc ttgtcagcat ggtctgcagg ttgtccaggt 10664

tggagtccat ggcgtccaag tggtcgctga gctcggtcct ggccagagga aggggagcag 10724

gtgacgtggc atccaggcac ccccaggtgc agtcctgccg gcccttaagc ccagctgcca 10784

gcctgccctg cgcaggctca cgggaggccc tgaggtgggg ccctgggcct gggacacttg 10844

gccaccggtg gtggctcagg ctccctcatc accgtgaggc cccgtaaggc agcacgtcag 10904

gctgggccgg ctaagacatc agggggctga gttcagggct cagaagggcc cggggcccca 10964

gcttctcagg ttcagcaacg cctgcccttc acactggagg acagcaggag ggtggagctg 11024

tgctcggcca ggctgggcca gcctacccca gcatgcgcca ggatagatta ggcctgcccc 11084

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cctgttcaca ggctgggcag gggtctgctc agaggctggg gaggggccca agctctggcg 11204

gagccctcct ccctccccag tgggacagcg ctaaccctgg ctggactcgg ccatgcagag 11264

aggaggaggg gcaggggaag aggcgggcga ccccagacat ctttggagtg cgagccaaac 11324

tgcaagatag aaagacaaga gccccccctct gcgggtgggg cccagcgagt ccaagccccc 11384

cacccgttc acccacctct ggggtggtag gacagacaga ccaggcggcc cggggagggc 11444

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ccccctggct ggcatgtctc cctccccagc tccatgctct gggccaggtc ggccagtttc 11564

ccccagcaaa tcccatgccg agggcctgag ggccatgcgt gtccagcccg gcactgtcct 11624

tgtcggaggc ccctgcgctt ccagagaccg aagggcgcct ggaaggcact cacttgtcta 11684

ggcaggcgac gctgaggcac ttctcgggag ccgaggcggg gagggtcgac ggatagcggc 11744

cccgagtgat ccgatagaag cttcgta 11771

FIGURE 2b continued

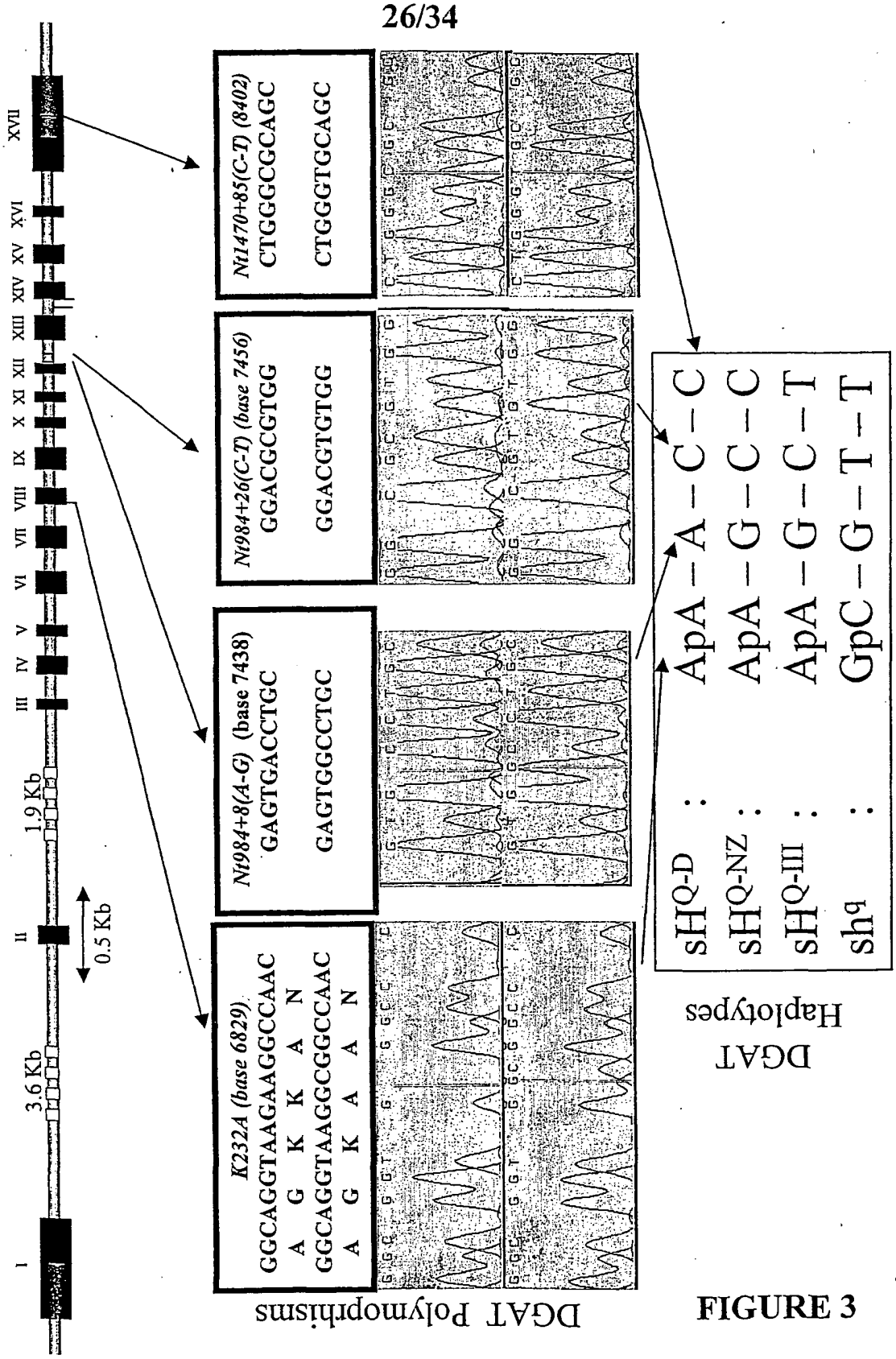

I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
3.6 Kb
1.9 Kb
0.5 Kb
K232A (base 6829).
GGCAGGTAAGAAGGCCAAC
A G K K A N
GGCAGGTAAGGCGGCCAAC
A G K A A N
Nt984+8(A-G) (base 7438)
GAGTGACCTGC
GAGTGGCCTGC
Nt984+26(C-T) (base 7456)
GGACGCGTGG
GGACGTGTGG
Nt1470+85(C-T) (8402)
CTGGGCGCAGC
CTGGGTGCAGC
DGAT Polymorphisms
DGAT Haplotypes
sHQ-D : ApA – A – C – C
sHQ-NZ : ApA – G – C – C
sHQ-III : ApA – G – C – T
sh9 : GpC – G – T – T

FIGURE 3

<221> VARIANT

<222> (232)..(232)

<223> an amino acid substitution (K -> A) caused by a polymorphism at bases 7224-7225 of the genomic sequence (measured from the adenine residue of the translation start codon). Lysine (K) corresponds to the Q allele, alanine (A) corresponds to the q allele.

<400> 5

```
Met Gly Asp Arg Gly Gly Ala Gly Gly Ser Arg Arg Arg Arg Thr Gly
1               5                  10                  15

Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
            20                  25                  30

Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
        35                  40                  45

Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
    50                  55                  60

Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser
65                  70                  75                  80

Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser
                85                  90                  95

Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val
            100                 105                 110

Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp
        115                 120                 125

Pro Ala Leu Cys Leu Val Ile Val Ala Asn Ile Phe Ala Val Ala Ala
    130                 135                 140

Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Ala
145                 150                 155                 160

Gly Leu Leu Leu His Gly Val Asn Leu Ala Thr Ile Leu Cys Phe Pro
                165                 170                 175
```

FIGURE 4a

```
Ala Ala Val Ala Phe Leu Leu Glu Ser Ile Thr Pro Val Gly Ser Val
            180                 185                 190

Leu Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr
        195                 200                 205

Arg Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala
    210                 215                 220

Lys Ala Ala Leu Ala Gly Lys Lys Ala Asn Gly Gly Ala Ala Gln Arg
225                 230                 235                 240

Thr Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe
                245                 250                 255

Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro
            260                 265                 270

Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Leu Leu Glu Met Leu Phe
        275                 280                 285

Leu Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met Val Pro Ala
    290                 295                 300

Ile Gln Asn Ser Met Lys Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile
305                 310                 315                 320

Val Glu Arg Leu Leu Lys Leu Ala Val Pro Asn His Leu Ile Trp Leu
                325                 330                 335

Ile Phe Phe Tyr Trp Leu Phe His Ser Cys Leu Asn Ala Val Ala Glu
            340                 345                 350

Leu Met Gln Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser
        355                 360                 365

Glu Ser Ile Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys
    370                 375                 380

Trp Cys Ile Arg His Phe Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser
385                 390                 395                 400

Lys Trp Ala Ala Arg Thr Ala Val Phe Leu Ala Ser Ala Phe Phe His
                405                 410                 415
```

FIGURE 4a continued

```
Glu Tyr Leu Val Ser Ile Pro Leu Arg Met Phe Arg Leu Trp Ala Phe
            420                 425                 430

Thr Gly Met Met Ala Gln Ile Pro Leu Ala Trp Ile Val Gly Arg Phe
        435                 440                 445

Phe Arg Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser Leu Ile Ile
    450                 455                 460

Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp Tyr Tyr Val Leu
465                 470                 475                 480

Asn Arg Glu Ala Pro Ala Ala Gly Thr
                485
```

FIGURE 4a continued

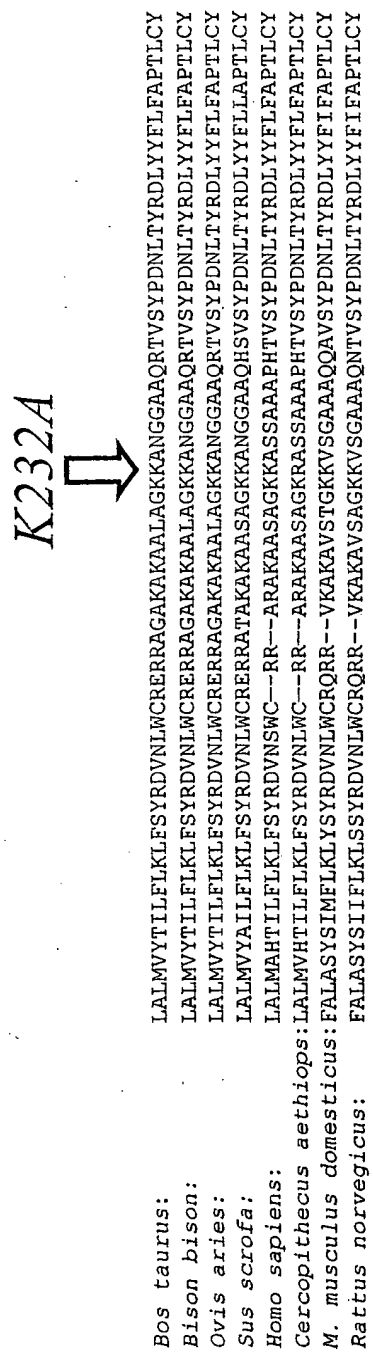
<400> 6

```
Met Gly Asp Arg Gly Gly Ala Gly Gly Ser Arg Arg Arg Arg Thr Gly
1               5                  10                  15

Ser Arg Pro Ser Ile Gln Gly Gly Ser Gly Pro Ala Ala Ala Glu Glu
            20                  25                  30

Glu Val Arg Asp Val Gly Ala Gly Gly Asp Ala Pro Val Arg Asp Thr
        35                  40                  45

Asp Lys Asp Gly Asp Val Asp Val Gly Ser Gly His Trp Asp Leu Arg
    50                  55                  60

Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp Ser Gly Phe Ser
65                  70                  75                  80

Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met Leu Ile Leu Ser
                85                  90                  95

Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr Gly Ile Leu Val
            100                 105                 110

Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp Pro Tyr Ser Trp
        115                 120                 125

Pro Ala Leu Cys Leu Val Ile Val Ala Asn Ile Phe Ala Val Ala Ala
    130                 135                 140

Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu Thr Glu Gln Ala
145                 150                 155                 160

Gly Leu Leu Leu His Gly Val Asn Leu Ala Thr Ile Leu Cys Phe Pro
                165                 170                 175

Ala Ala Val Ala Phe Leu Leu Glu Ser Ile Thr Pro Val Gly Ser Val
            180                 185                 190

Leu Ala Leu Met Val Tyr Thr Ile Leu Phe Leu Lys Leu Phe Ser Tyr
        195                 200                 205

Arg Asp Val Asn Leu Trp Cys Arg Glu Arg Arg Ala Gly Ala Lys Ala
    210                 215                 220

Lys Ala Ala Leu Ala Asp Leu Tyr Tyr Phe Leu Phe Ala Pro Thr Leu
225                 230                 235                 240
```

FIGURE 4b

```
Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro Arg Ile Arg Lys Arg Phe
                245                 250                 255

Leu Leu Arg Arg Leu Leu Glu Met Leu Phe Leu Thr Gln Leu Gln Val
            260                 265                 270

Gly Leu Ile Gln Gln Trp Met Val Pro Ala Ile Gln Asn Ser Met Lys
        275                 280                 285

Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile Val Glu Arg Leu Leu Lys
    290                 295                 300

Leu Ala Val Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu
305                 310                 315                 320

Phe His Ser Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp
                325                 330                 335

Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser Glu Ser Ile Thr Tyr Phe
            340                 345                 350

Trp Gln Asn Trp Asn Ile Pro Val His Lys Trp Cys Ile Arg His Phe
        355                 360                 365

Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser Lys Trp Ala Ala Arg Thr
    370                 375                 380

Ala Val Phe Leu Ala Ser Ala Phe Phe His Glu Tyr Leu Val Ser Ile
385                 390                 395                 400

Pro Leu Arg Met Phe Arg Leu Trp Ala Phe Thr Gly Met Met Ala Gln
                405                 410                 415

Ile Pro Leu Ala Trp Ile Val Gly Arg Phe Phe Arg Gly Asn Tyr Gly
            420                 425                 430

Asn Ala Ala Val Trp Leu Ser Leu Ile Ile Gly Gln Pro Val Ala Val
        435                 440                 445

Leu Met Tyr Val His Asp Tyr Tyr Val Leu Asn Arg Glu Ala Pro Ala
    450                 455                 460

Ala Gly Thr
465
```

FIGURE 4b continued


K232A
Bos taurus: LALMVYTILFLKLFSYRDVNLWCRERRAGAKAKAALAGKKANGGAAQRTVSYPDNLTYRDLYYFLFAPTLCY
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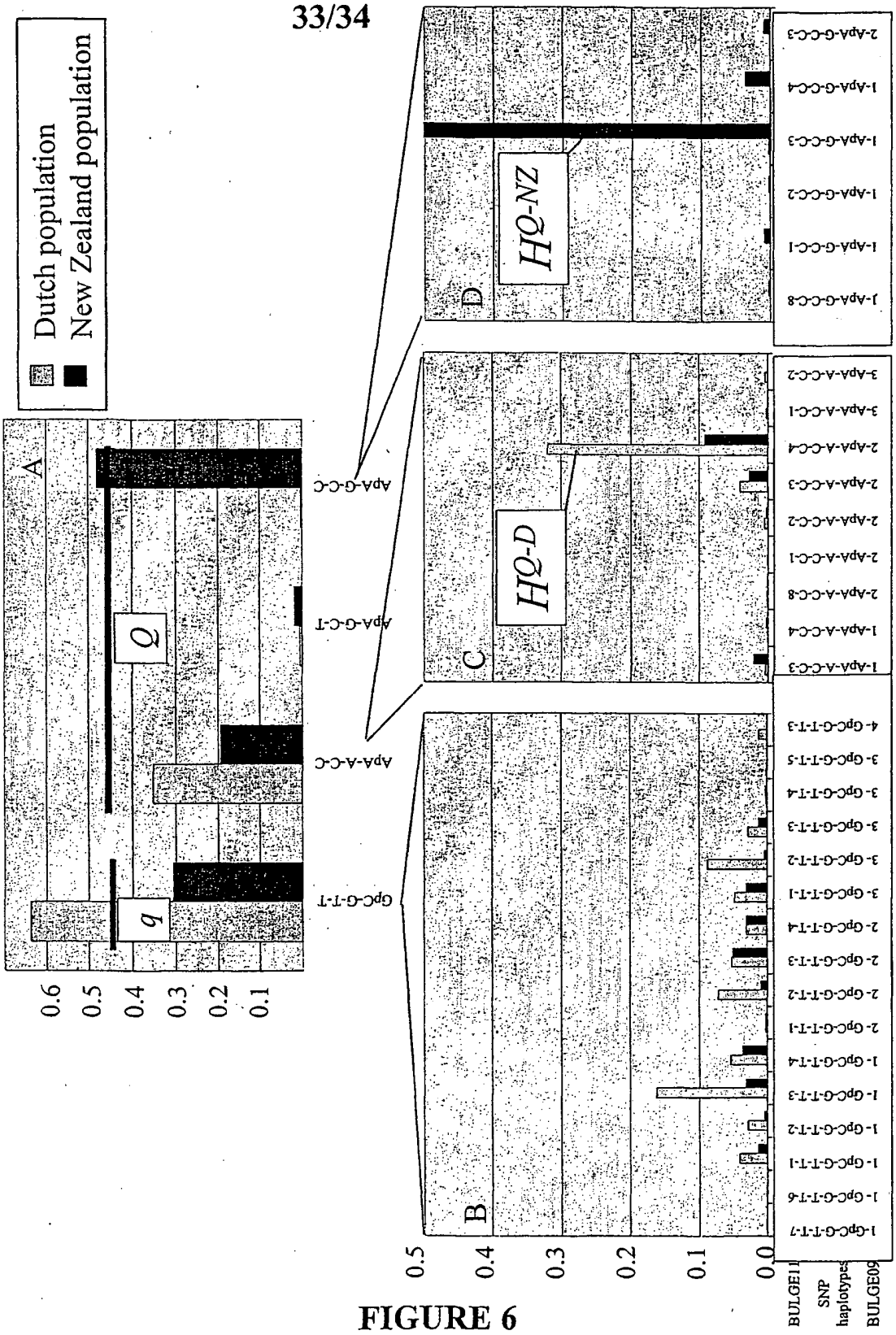
Ovis aries: LALMVYTILFLKLFSYRDVNLWCRERRAGAKAKAALAGKKANGGAAQRTVSYPDNLTYRDLYYFLFAPTLCY
Sus scrofa: LALMVYAILFLKLFSYRDVNLWCRERRATAKAKAASAGKKANGGAAQHSVSYPDNLTYRDLYYFLLAPTLCY
Homo sapiens: LALMAHTILFLKLFSYRDVNSWC--RR--ARAKAASAGKKASSAAAPHTVSYPDNLTYRDLYYFLFAPTLCY
Cercopithecus aethiops: LALMVHTILFLKLFSYRDVNLWC--RR--ARAKAASAGKRASSAAAPHTVSYPDNLTYRDLYYFLFAPTLCY
M. musculus domesticus: FALASYSIMFLKLYSYRDVNLWCRQRR--VKAKAVSTGKKVSGAAAQQAVSYPDNLTYRDLYYFIFAPTLCY
Rattus norvegicus: FALASYSIIFLKLSSYRDVNLWCRQRR--VKAKAVSAGKKVSGAAAQNTVSYPDNLTYRDLYYFIFAPTLCY

FIGURE 5


Dutch population
New Zealand population
A
Q
q
ApA-G-C-C
ApA-G-C-T
ApA-A-C-C
GpC-G-T-T
0.6
0.5
0.4
0.3
0.2
0.1
B
C
D
HQ-D
HQ-NZ
1-GpC-G-T-T-7
1-GpC-G-T-T-6
1-GpC-G-T-T-1
1-GpC-G-T-T-2
1-GpC-G-T-T-3
1-GpC-G-T-T-4
2-GpC-G-T-T-1
2-GpC-G-T-T-2
2-GpC-G-T-T-3
2-GpC-G-T-T-4
3-GpC-G-T-T-1
3-GpC-G-T-T-2
3-GpC-G-T-T-3
3-GpC-G-T-T-4
3-GpC-G-T-T-5
4-GpC-G-T-T-3
1-ApA-A-C-C-3
1-ApA-A-C-C-4
2-ApA-A-C-C-8
2-ApA-A-C-C-1
2-ApA-A-C-C-2
2-ApA-A-C-C-3
2-ApA-A-C-C-4
3-ApA-A-C-C-1
3-ApA-A-C-C-2
1-ApA-G-C-C-8
1-ApA-G-C-C-1
1-ApA-G-C-C-2
1-ApA-G-C-C-3
1-ApA-G-C-C-4
2-ApA-G-C-C-3
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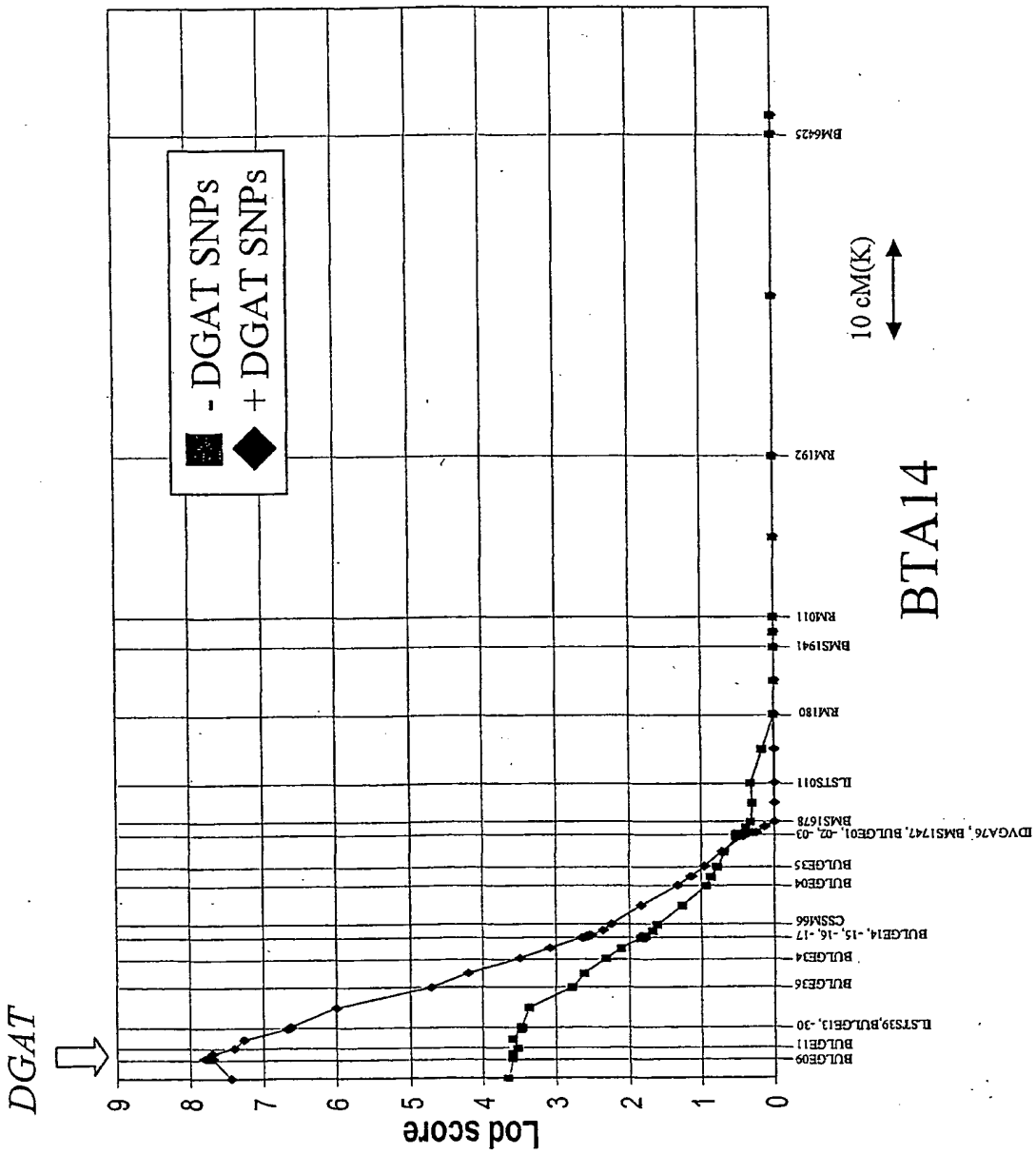
0.4
0.3
0.2
0.1
0.0
BULGE11
SNP haplotypes
BULGE09

FIGURE 6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+ DGAT SNPs
10 cM(K)
BTA14
DGAT
lod score
0
1
2
3
4
5
6
7
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9
BM6425
RM192
RM011
BMS1941
RM180
ILSTS011
BMS1678
IDVGA76, BMS1747, BULGE01, -02, -03
BULGE35
BULGE04
CSSM66
BULGE14, -15, -16, -17
BULGE34
BULGE36
ILSTS39,BULGE13, -30
BULGE11
BULGE09

FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00245

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12Q 1/68, C12N 15/12, A01K 67/027, C07K 16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

WPIDS, CA: SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AGRICOLA, BIOSIS, DNA DATABASES (GENBANK, EMBL): SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS, GENBANK, EMBL, BIOSIS, CA, MEDLINE, AGRICOLA: diacylglycerol acyltransferase, dgat1, bovine, cow, cattle, milk

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Accession No AF059202 15 October 1998 Oelkers P et al *Homo sapiens* ACAT related gene product 1 mRNA, complete cds	27-33, 35-37
X	EMBL Accession No AF236018 12 August 2000 Joyce CW et al *Cercopithecus aethiops* diacyl-glycerol acyltransferase mRNA, complete cds	27-33, 35-37

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2002	13 FEB 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **TERRY MOORE** Telephone No : (02) 6283 2632

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00245

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Accession No AF078752 11 November 1998 Cases S et al *Mus musculus* diacylglycerol acyltransferase (Dgat) mRNA, complete cds	27-33, 35-37
X	Smith CJ et al "Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat" (2000) Nature Genetics 25(1), pages 87-90 See the whole document.	44-46

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00245

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **1-50 in part and claims 7 and 14-22 in full.**

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See the explanation in the supplemental box.

3. ☐ Claims Nos :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00245

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: Box I(2)

Claims 1-50 have not been searched in full because the applicant, although invited to furnish the sequence listing in computer readable form, failed to do so. As such the claims were not searched with regard to the sequences disclosed in the specification. The claims have simply been searched using prior art human and mouse DGAT1 gene sequences located in the EMBL and GENBANK databases.

Claims 7 and 14-22 have not been searched in full because the scope of the claims is unclear. The claims include within their scope bovines that are not the products of the invention and whose pre-existing genotype is simply further characterised using the DGAT1 gene. It is impossible to determine which bovines would fall within the scope of these claims and as such a meaningful search cannot be conducted.